GROWTH AND PHYSIOLOGICAL PARAMETERS RELATED TO SHOOT DIEBACK IN *Pterocarpus angolensis* DC SEEDLINGS

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DECLARATION

I, Jacob P. Mwitwa, do hereby declare that the contents of this dissertation are the product of my work and have not been submitted previously at any university for a degree. All references to previously published and unpublished content have been duly acknowledged.

Signed

Date

05/03/2004

SUMMARY

Six experiments, five in the glasshouse and one in the field near Nelspruit, were carried out to ascertain the effect of factors related to shoot die-back, and of water treatments on the growth and physiological responses of *Pterocarpus angolensis* seedlings. The study was undertaken to broaden the knowledge and understanding of the phenomenon of shoot die-back in order to enhance our ability to regenerate the species. The following experiments were carried out

(a) *Assessment of biomass accumulation; anatomical characteristics of the shoot apical meristem; foliar, stem and root concentration of micro- and macro-nutrients associated with each phenophase,*

(b) *Effect of seedling age and seed source on the occurrence of shoot die-back under field conditions;*

(c) *Water treatment effects on Chl. a fluorescence traits of P. angolensis seedlings obtained by assessing the fluorescence yield of photosynthetic samples subjected to dark- and light-adaptation;*

(d) *Genetic variation in shoot die-back and other traits of sixteen half-sib families of P. angolensis from Malawi, Namibia and Zambia grown over two die-back seasons.*

Experiments conducted revealed the following

1. *Patterns of growth observed in phenophases are indicators of seasonal changes in annual biomass allocation to the shoot and root. Phenophases such as leaf loss and stem senescence, whether shoot die-back occurs completely or not, are directly related to the decline in above-ground biomass and declined rate of increase in root biomass respectively. Leaf flush, expansion and maturation result in increased biomass accumulation whilst shoot die-back has a minimal down-regulatory effect on root biomass accumulation compared to the shoot. Shoot die-back is not sudden, therefore from the first day of germination, seedlings synchronise growth and development with the occurrence of shoot die-back.*

2. *Phenophasic concentration of foliar N, Ca and Mg, stem Fe and Cu and root concentrations of P, K, Mg, Fe and B are associated with shoot die-back. Patterns of mineral nutrient concentration obtained in foliage and roots but to a lesser extent in the stem, may be related to nutrient remobilisation during shoot die-back. Higher relative mineral nutrient changes during leaf yellowing and shoot die-back may be an indication of the removal of significant volumes of mobile nutrients from senescing tissues.*

3. *The volume of the shoot apex of P. angolensis remains constant during different phenophases which points to seasonal uniformity in the size of the apical dome. Changes in phenology associated with declined growth, or shoot die-back, is revealed through declined cell number in the tunica which is a reflection of declined mitotic activity.*

4. *Shoot die-back occurs in all seedlings from nursery stock planted under field conditions and all seedlings of up to two years experience complete shoot die-back. Shoot die-back takes place irrespective of seed source or the age of nursery stock that is planted. Survival after the first shoot die-back is normally low. Water treatments had no significant effect on the function of PSII reaction centres of P. angolensis nursery seedlings. In the case of both dark- and light-adapted leaves, water treatment had no significant effect on the measured Chl. a fluorescence parameters or the calculated parameters (specific activities, phenomenological fluxes, structure-function and performance indexes and driving forces).*

5. *Water treatments affect the shape of Chl. a fluorescence transients of light-adapted compared to that of dark-adapted photosynthetic samples of P. angolensis. No significant water treatment effect was obtained for extracted and technical Chl. a fluorescence parameters, specific fluxes, quantum efficiencies and phenomenological fluxes. Quantum yield, relative electron transport and quantum yield limitation, de-excitation rate constants, structure-function, performance indexes and driving forces were also not significantly different across water treatments.*

6. *Genetic variation was observed to exist among 16 half-sib families from Malawi, Namibia and Zambia. High heritabilities were obtained for shoot die-back and other traits, indicating that shoot die-back is genetically controlled. The trait is passed from parents to offspring and it is highly probable that it occurs, throughout its natural range, in all seedlings. Since shoot die-back is genetically programmed, it remains crucial to the ability of a seedling to regenerate in the following rainy season.*

OPSOMMING

Ses eksperimente, vyf in die glashuis en een in die veld naby Nelspruit, is uitgevoer om die effek van faktore wat verwant is aan die terugsterwing van lote op saailinge, sowel as om die effek van waterstres op die groei en fisiologiese responsies van *Pterocarpus angolensis* saailinge, te ondersoek. Die studie is onderneem om die kennis en begrip aangaande die regenerasie-dinamika van die spesies te verbeter. Die volgende eksperimente is uitgevoer:

(a) *Evaluering van die effek van fisiologiese veranderings op biomassa; blaar-, stam- en wortelkonsentrasies van spoor- en makro-voedingselemente, en anatomiese eienskappe van die apikale meristeem van die lote.*

(b) *Effek van saailingouderdom en saadbron op die voorkoms van loot-terugsterwing onder veldtoestande.*

(c) *Waterbehandelingseffekte op Chl. a fluorisensie eienskappe van* P. angolensis *saailinge wat verkry is deur die fluorisensie te evalueer van fotosintese-monsters wat aan donker- en lig-adaptasies onderwerp is.*

(d) *Genetiese variasie in loot-terugsterwing en ander groei-eienskappe van 16 halfsib families van* P. angolensis *vanaf Malawi, Namibia en Zambia wat gekweek is oor twee terugsterf-seisoene.*

Die eksperimente het die volgende aan die lig gebring:

1. *Groeipatrone waargeneem gedurende die fenofases is indikatore van seisoenale veranderings in jaarlikse biomassa allokasies aan die loot en die wortels. Fenofases soos blaarverlies en lootafsterwing, ongeag of loot-terugsterwing volledig is of nie, is direk verwant aan die afname in bogrondse biomassa en afnemende tempo van toename in wortelbiomassa respektiewelik. Bottende blare, vergroting en rypwording van blare lei tot toenemende biomassa akkumulasie terwyl loot-terugsterwing 'n minimale afskalende effek op akkumulasie van wortelbiomassa het in vergelyking met die van die loot. Loot-terugsterwing is nie skielik, met ander woorde vanaf die eerste dag van ontkieming sinchroniseer saailinge groei en ontwikkeling met die voorkoms van loot-terugsterwing.*

2. *Fenofase konsentrasies van en veranderings in blaar N en Ca en loot Fe, asook veranderings in waargenome wortel N, K, Ca, Mn, Cu, Zn en B is sterk ge-assosieer met loot-terugsterwing. Patrone van minerale voedingselementkonsentrasies wat in blare en wortels, en in minder mate in die loot, verkry is, mag direk verwant wees aan hermobilisering van voedingselemente gedurende loot-terugsterwing. Hoë relatiewe minerale voedingselementveranderings gedurende die vergeling van blare en loot-terugsterwing mag 'n indikasie wees van die verwydering van betekenisvolle hoeveelhede mobiele nutriente vanaf sterwende weefsel.*

3. *Die volume van die groeipunt van* P. angolensis *bly konstant gedurende verskillende fenofases wat dui op seisoenale uniformiteit in die grootte van die apikale koepel. Veranderings in fenologie ge-assosieer met afnemende groei, of loot-terugsterwing, word gereflekteer deur afnemende selgetalle in die tunika wat dui op afnemende mitotiese aktiwiteit.*

4. *Volledige loot-terugsterwing kom voor in alle saailinge vanaf die kwekery wat in die veld geplant word tot op die ouderdom van twee jaar. Dit kom voor angeag van saadbron of ouderdom van saailinge ten tye van planting. Oorlewing na aanvanklike loot-terugsterwing is normaalweg laag.*

5. *Water behandelings het geen beduidende effek op die funksie van PSII reaksiesentra van* P. angolensis *kewekery-saailinge gehad. Vir beide donker- en lig-aangepaste blare is geen beduidende waterbehandelingseffek verkry vir waargenome Chl. a fluoresensie parameters of die berekende parameters (spesifieke aktiwiteite, fenomenologiese flukse, struktuur-funksie-indekse, "performance-indekse of "driving forces").*

6. *Genetiese variasie tussen 16 halfsibfamilies vanaf Malawi, Namibie en Zambie is verkry vir loot-terugsterwing en ander groei-eienskappe. Dit dui op genetiese beheer van terugsterwing en dat die eienskap oorerfbaar is, en waarskynlik in die hele natuurlike verspreidingsgebied van die spesies in alle saailinge voorkom. Aangesien loot-terugsterwing geneties geprogrammeer is, is dit noodsaaklik vir die vermoë van die plant om in die volgende reënseisoen te regenereer.*

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To

Priscilla Chipendano,
Bridget Mwitwa, Mwengwe Mwitwa, Chibeka C. Mwitwa, Shandinshiku C. J. Mwitwa.

An acknowledgement of hope and trust effuses from the depth of my conscience that has amplified my cherished belief in the importance of the family and hope based on trust. You have all borne the burden of loneliness that bordered on abandonment, but never was a line in this thesis written without thinking about how special each one of you was to me and this work. Simply because you were and are still a part of me. I dedicate this work to you all hoping that you will accept my sincere thoughts. I cannot undo the hours of loneliness or the years my children have gone without the presence of a father, but can only explain the reasons of my absence in these few lines. Not a day went by that I never thought of you all. There is a picture in my heart and a memory in my soul for each one of you. May the love and grace of Jesus our Lord continue to magnify you and your endeavours.

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CHAPTER 1

1. INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Pterocarpus angolensis DC, a member of the *Fabaceae* family (syn. *Papilionaceae*) which is a subfamily of *Leguminosae* occurs in Angola, DR Congo, Malawi, Mozambique, Namibia, Tanzania, South Africa, Zambia and Zimbabwe (Fig. 1.1). *Pterocarpus angolensis* is popularly known as bloodwood (English), bloedhout, bastergreinhout or kiaat (South Africa), mukwa or mulombwa in other African countries in which it is found. The species covers a diverse and varied ecological zone, occurring on sandy soils of low nutrient status, and high pH, and prefers alkaline, light textured and free drainage soils (Boaler, 1966; Vermeulen, 1990). Together with the varied soil types, the area of occurrence is also diverse in mean annual rainfall (500 mm to 1200 mm) and mean annual temperature (22^{o}C to 35^{o}C). Associated species vary with regard to climatic and soil conditions. In the south-east of Angola, north eastern Namibia and south western Zambia, *P. angolensis* is associated with species of the Zambezi teak forests whereas in the high rainfall regions, it is associated with species specific to the miombo woodlands (*Brachystegia, Isoberlinia* and *Julbernadia*).

The species is considered to be one of the best known and valuable hardwood tree species in eastern, central and tropical southern Africa (Coates-Palgrave, 1983; Vermeulen, 1990; van Daalen *et al.*, 1992; Stahle *et al.,* 1999). The wood is easy to work and is therefore used in carvings, furniture, joinery, veneer and as a general-purpose timber (Coates-Palgrave, 1983; Vermeulen, 1990; Van Daalen *et al.*, 1992; Stahle *et al.,* 1999). The value of the species, which includes medicinal uses, has made it one of the most sought after tropical hardwood species by local communities and commercial logging companies. *P. angolensis* is considered an endangered species due to poor stewardship and long-term adverse climatic conditions (Munyanziza and Oldeman, 1995; Musokonyi, 1998 and Stahle *et al.*, 1999).

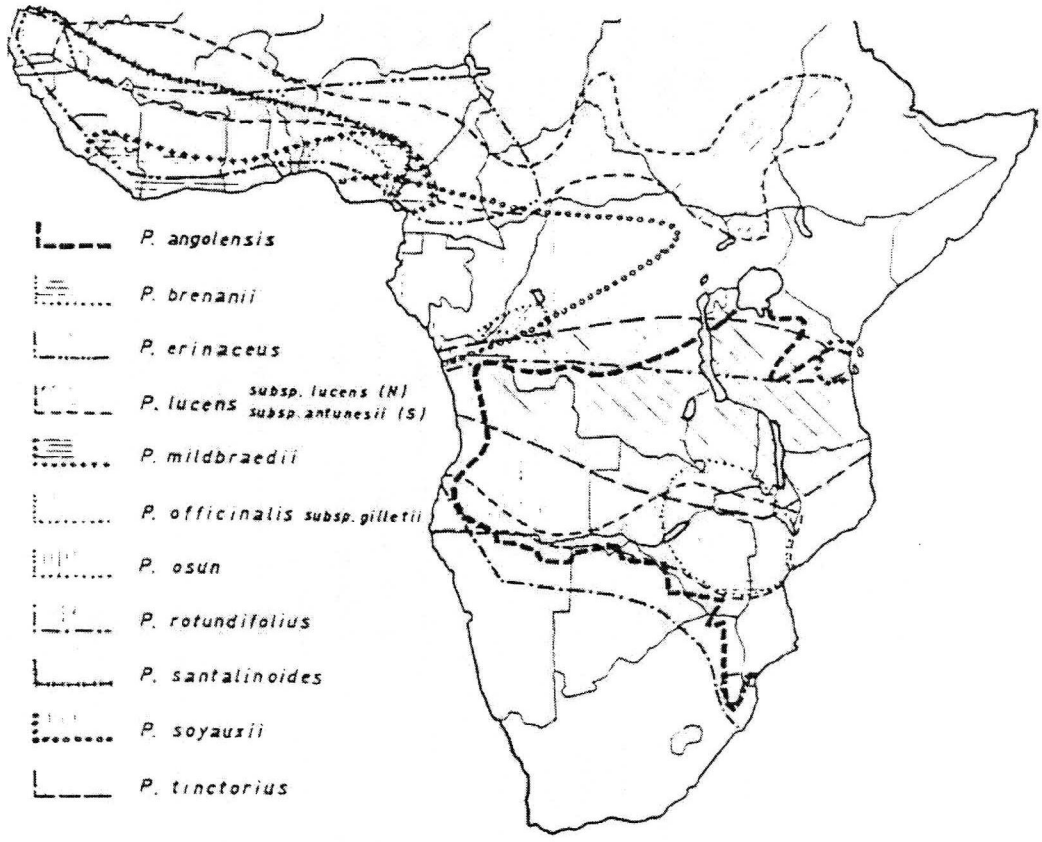

Figure 1.1. Distribution map of the genus Pterocarpus in Africa. (Von Breitenbach, 1973).

Problems related to stewardship include inappropriate inventory procedures (Stahle *et al.*, 1999), unknown natural regeneration dynamics, non-existent and inappropriate silvicultural technology (Munyanziza and Oldeman, 1995) and forest management methods specific to ecosystems in which the species occurs. Natural regeneration of the species in the miombo woodlands is greatly affected by stressful environmental conditions such as the long dry season, annual fires and in some areas by browsing (Munyanziza and Oldeman, 1995; Stahle *et al.,* 1999). The single rainy season, interspersed with a long dry season, plays a very important role in the natural regeneration cycles of the species. The moisture conserved by the pods during the dry season attracts termites that crack the hull (Graz, 1996). Severe fires occuring in the dry season then burn or scorch the pod so that the germinating seed easily breaks through during the rainy season. *P.angolensis* is sensitive to overshadowing, therefore high natural regeneration rates are apparent mainly in open woodlands or open areas of closed forests. According to Graz (1996) and Kasumu (1998), the species germinates best in the glasshouse when it is not shaded.

Grazing and browsing impact negatively on the ability of the species to regenerate *in situ.* In *in situ* conservation stands of the Zambezi teak forests in south-western Zambia, more than 70% of planted seedlings are browsed and rarely survive in the subsequent growing season.

Research has been conducted on seed germination (van Daalen, 1981; Kasumu, 1998; Musokonyi, 1998), genetic variation in seedling growth in the glasshouse (Munthali, 1999) and root pruning and fertilisation in the glasshouse (Munyanziza and Oldeman, 1995; Munyanziza *et al.*, 1998). *P.angolensis* ecology (Boaler, 1966), population dynamics in permanent sample plots (for stem counts, height and diameter at breast height), fire ecology and land use (Graz, 1996) and management implications on annual growth rings (Stahle *et al.,* 1999) have also been carried out. A condition generally called the *suffrutex* (Boaler, 1966) in which the shoot dies back to about 3 cm below ground each dry season has been reported and assumed to be the major hindrance to the successful regeneration and ageing of *P.angolensis*.

Due to its less understood growth characteristics, *P.angolensis* has not been extensively planted from seedlings or vegetative material under plantation conditions despite its high value for joinery and other uses.

1.1.1 Problem Statement

The *suffrutex* phenomenon (Boaler, 1966; Vermuelen, 1990; Graz, 1996; Kasumu, 1998) in which the shoot dies back to about 3 cm below ground each dry season, for up to seven years before the sapling stage (Menaut *et al.*, 1995), has been reported to be a common and necessary process in the development of the species. Natural regeneration is significantly influenced by this physiological process because of the fact that bush fires sometimes burn even the part of the shoot that lies below the surface close to the live root. Whether annual fires do or do not affect the root and the subsequent shoot development in the following growing season is unknown. It is generally assumed that the purpose of this developmental habit is to re-allocate all resources to the development of the taproot, which swells and tapers from the root collar downwards, probably as a storage organ.

It has not been established whether this type of root is a lignotuber and is principally developed for the storage of water or not. It has been indicated by Munyanziza and Oldeman (1995) that the swollen taproot is a storage organ for energy reserves. Seedlings, 245 days of age, which were found by Kasumu (1998) to have developed root biomass five times that of the shoot partly confirms the observation of several authors with respect to the low shoot to root ratio in the seedling (suffrutex) stage. In situations where nitrogen and phosphorus are applied, biomass allocation has been found to be in favour of the shoot (Munyanziza and Oldeman, 1995). The probable factors influencing this disparity and phenomenon of shoot die-back have not been elucidated, whether it is a single factor or a combination of environmental and/or physiological factors that are involved, are still unknown.

Previous research by Boaler (1966), Munyanziza and Oldeman (1995) and Kasumu (1998), have not shown sequential or periodic increases or decreases in the biomass which could have given an indication of the relationship between biomass increment and time in relation to changes in phenology. There are no reports of research that have determined changes in phenotypic and physiological traits in relation to changes in environmental conditions. The results of such work could have assisted in understanding the effects of the plant's external environmental conditions on the physiological development, particularly the phenomenon of shoot die-back. The absence of information on the natural regeneration dynamics and environmental effects results in poor silvicultural regimes and a general lack of interest in artificial regeneration.

The present work therefore focuses on identifying growth and physiological parameters related to shoot die-back in *P. angolensis* seedlings. The parameters that were studied are the trend of biomass allocation, phenophasic changes in mineral nutrient concentration, changes in the anatomy of the shoot apex associated with phenophases, establishment of the existence of family variation in shoot die-back and identification of whether different levels of water supply impose physiological stress on *P. angolensis*, with particular emphasis on chlorophyll-*a* fluorescence properties of treated seedlings.

1.1.2 Aim and objectives

1.1.2.1 Ultimate aim of the study

The research was carried out to improve our understanding of the phenomenon of shoot die-back in *Pterocarpus angolensis* that will subsequently aid in understanding the dynamics of seedling development in order to enhance our ability to regenerate the species.

1.1.2.2 Proximate objectives

- To determine biomass allocation in *P. angolensis* associated with phenophases (i.e. changes in the phenology of seedlings) that include shoot die-back;
- To determine phenophasic changes in the anatomy of the shoot apex that may be associated with shoot die-back;
- To ascertain the existence of phenophasic mineral nutrient patterns in the foliage, stem and root;
- To determine the effect of seedling age and seed sources of nursery stock and direct seeding on the occurrence of shoot die-back.
- To assess the effect of water treatments on chlorophyll-*a* fluorescence properties of *P. angolensis* seedlings;
- To ascertain whether shoot die-back has a genetic basis or not.

1.2 LITERATURE REVIEW

1.2.1 The senescence of plant shoots

The suffrutex phenomenon reported in *P. angolensis* seedlings (Boaler, 1966; Vermeulen, 1990; Graz, 1996; Kasumu, 1998) mainly affects the shoot. The suffrutex phase refers to the sequential growth, death and re-growth of the seedling shoot. Due to this recurring physiological behaviour, seedlings do not grow above the shrub layer during the first 10 years. The phenomenon is brought about by the seasonal death of the shoot to about 3 cm below ground each dry season and re-growth from buds on the upper parts of the tuber in the following rainy season. The sequence of shoot death has been referred to as shoot die-back and has been assumed to last up to 10 years before the seedling can produce a shoot that will break out of the suffrutex phase and grow into the final tree trunk.

Shoot die-back in *P. angolensis* can be classified as a classical form of organ senescence. Patterns of senescence seem to differ in different important ways, both in their causes and the nature of the processes itself that include the degree of reversibility (Bidwell, 1979). Senescence is a developmental process which is highly ordered and programmed. It can involve the death of the whole plant at the same time, progressive senescence of plant organs as the whole plant ages or senescence of only a plant's organ (Trippi, 1990).

As a normal process in the development of plants, senescence can involve individual cells only, entire plant organs or the whole plant (Campbell, 1993). At the level of cells, tracheids and cork cells normally age and die before becoming fully specialised and functional. These processes ultimately lead to the complete loss of organisation and function of plant cells.

Patterns of senescence

Organ senescence was observed by Noodén (1988a) and Campbell (1993) to follow distinct patterns of senescence in different plant organs such as leaves and shoots. A range of patterns exists in leaves with the main ones being progressive senescence in which the older leaves senesce as new leaves are produced, or all the leaves senescing together (Noodén, 1988a; Taiz and Zeiger, 1998). Also, leaf senescence may synchronously affect all the leaves on a seasonal basis (Lambers *et al.,* 1998). Even though leaf senescence may also be induced by disease or environmental cues, the correlative and environmental regulating mechanisms of the senescence patterns may be different but the fundamental metabolic aspects may not necessarily be different (Noodén, 1988a; Lambers *et al.,* 1998; Taiz and Zeiger, 1998). The senescence of the aerial portion, which is also referred to as top senescence, occurs in many terrestrial plants for the purpose of survival during adverse conditions (Bidwell, 1979).

When this occurs, the plant may retreat to an underground root or stem system until the time of favourable environmental conditions (Noodén, 1988a; Campbell, 1993). Cells within a leaf may senesce at different times and the parts of the same organ may also senesce at different times (Salisbury and Ross, 1985; Taiz and Zeiger, 1998).

In some species, leaf yellowing begins with the main vein, called vein yellowing, and later spreads to the intervenal regions whereas in other species leaf senescence starts from the apex and spreads toward the base (Noodén, 1988a). Surface hairs precede the leaf blade during senescence with the tissues around the vascular bundle senescing later. Stomatal guard cells, due to a lack of symplastic connection with neighbouring cells, live far beyond the senescence of other leaf cells.

When leaves are about to abscise, the abscission layer, which in this case is also the breaking point, is located near the petiole (Salisbury and Ross, 1985; Campbell, 1993; Taiz and Zeiger, 1998). The cells of the abscission layer are small thin-walled parenchyma cells (Salisbury and Ross, 1985), and exhibit an absence of fibre cells around the vascular bundles (Campbell, 1993).

The enzymatic hydrolysis of the cell wall, middle lamella, and polysaccharides of the abscission layer further weakens the abscission layer, which, with the added weight of the leaf causes a separation of the senesced leaf from the plant. It has also been reported by Salisbury and Ross (1985) that the digestion of the middle lamella involves the synthesis of polysaccharide-hydrolysing enzymes, mainly cellulases and pectinases, and their secretion from the cytoplasm into the cell wall.

Causes of senescence

The phenomenon of senescence can be triggered by internal factors, as in monocarpic senescence, or external factors such as shortening days and temperature (Campbell, 1993) and by water deficit or drought (Itai and Benzioni, 1976; Taiz and Zeiger, 1998; Rood *et al*, 2000). One or a combination of the foregoing can then lead to an ordered sequence of cytological and biochemical events. Senescence is a natural developmental process, which may be completely endogenous and can be thought of as terminal differentiation (Bidwell, 1979; Noodén, 1988a).

In such a case therefore, natural causes of senescence in cells, tissues, organs and organisms can be caused by endogenously controlled deteriorative changes. The aforementioned changes are brought about by changes in chemical, metabolic and other physiological factors, with each factor responding differently to the environment (Pereira, 1994). Important aspects identified by Pereira (1994) with regard to the plant's response to the environment, i.e. short-term responses, acclimatisation to new environmental conditions and survival, imply very different metabolic and regulatory responses.

Enzymatic Activity

Organ senescence starts with catabolic processes that are preceded by the synthesis of several hydrolytic enzymes that include chlorophyll-degrading enzymes, lipases, nucleases and proteases (Taiz and Zeiger, 1998). Therefore, the senescence and death of the cell is an active process and an orderly loss of normal cellular functions.

The cellular changes that take place during organ senescence have been grouped into central and peripheral changes (Noodén, 1988b) with both forming a syndrome of physiological changes associated with organ senescence. Senescence begins with relatively small quantitative changes in regulatory senescence gene expression due to the fact that the internal programming of senescence implies that it is genetically controlled (Noodén, 1988b; Trippi, 1990; Taiz and Zeiger, 1998). This is due to the fact that the form and organization of plants depend on the genetic constitution of a plant (Trippi, 1990). An alteration in the synthesis of RNA and protein occurs early in the senescence process whereas the selective activation of the synthesis of some mRNAs and proteins may be the most likely initiation of active cellular processes leading to senescence. Other changes include decreases in DNA, inorganic ions and various organic nutrients (Bidwell, 1979). A significant diminution in the levels of most chloroplast mRNAs (Sabater *et al.,* 1990b) and an increase in the abundance of certain transcripts (Taiz and Zeiger, 1998) occur during senescence occur.

Senescence genes

Senescence down-regulated genes (SDGs) are genes whose expression decreases during senescence and include genes encoding proteins that are involved in the process of photosynthesis. Genes that encode hydrolytic enzymes, such as proteases and ribonucleases, and enzymes involved in ethylene biosynthesis, such as *l*-aminocyclopropane-*l*-carboxylic acid synthases and oxidases, are called senescence-associated genes (Taiz and Zeiger, 1998). Working with barley (*Hordeum vulgare* L. cv. Hassan) senescent chloroplasts, Sabater *et al.* (1990b) discovered a steeper decline in the level of Ba2 transcripts of senescent leaves than is common at other stages of leaf development.

Genes for senescence polypeptides were found to map in the single short chain of chloroplast DNA instead of the single large and inverted repeat chains associated with plastid greening and leaf growth. Gene expression is controlled by cytokinins as one of the mechanisms through which cytokinins retard senescence of leaves (Sabater, *et al.,* 1990a). Even though some mRNAs for senescence polypeptides remain for sometime in chloroplasts, a strong translational control inhibits their use as templates in protein synthesis in the chloroplasts of cytokinin-treated leaves.

Genes expressed during senescence include genes with secondary functions that encode enzymes involved in either the conversion or remobilisation of breakdown products (Sabater *et al.*, 1990b; Taiz and Zeiger, 1998). The breakdown products of senescence include glutamine and glutamate synthetase which catalyse the conversion of ammonium into glutamine and nitrogen recycling from senescing tissues (Taiz and Zeiger, 1998). Glutamine synthetase found in the cytosol produces glutamine for intracellular nitrogen transport and that located in shoot chloroplasts reassimilates photorespiratory NH_4^+. A general feature of senescing organs is an increase in glutamate dehydrogenase whose *de novo* synthesis is regulated by ammonia produced by proteolysis (Lettgen *et al.*, 1990). The change in gene expression is followed by changes in swelling of thylakoids and the appearance of lipid droplets and plastoglobuli, and the likely loss of ribosomes from the cytoplasm and stroma of chloroplasts (Thompson, 1988).

The endpoint of early changes include loss of the integrity of lipid bilayers of plasma and microsomal membranes (Thompson, 1988) and late changes in mitochondrial and vacuolar membranes which are followed by an autophagic process where cell organelles are enveloped in vacuole-like structures (Noodén, 1988a). Concurrent with bilayer destabilisation (i.e. rigidification of bulk membrane lipids, formation of gel phase lipid and nonbilayer lipid configuration) is the manifestation of membrane leakiness, advanced proteolytic activity and generalised loss of membrane function (Thompson, 1988). Membrane deterioration that leads to the loss of intracellular compartmentation results in the aforementioned membrane leakiness whose manifestation is proven by the leakage of pigments, sugars and electrolytes. The breakdown of the nucleus which may occur relatively early in xylem cell differentiation is a late event of ultrastructural changes.

Thylakoid membrane senescence

Chloroplast structures undergo deteriorative changes soon after leaf expansion has been accomplished and these begin with symptoms of deterioration in the energy-transducing thylakoid membranes much before changes occur in the envelope membranes (Thompson, 1988).

The general trend of thylakoid membrane senescence involves free radical production and the loss of thylakoid membrane integrity (Thompson, 1988; Benson, 1990). Studies in *Phaseolus vulgaris* have shown a four-fold increase in the formation of superoxide radical (O^{-}_2) by illuminated thylakoids during early stages of senescence which decline with the intensity of senescence (McRae and Thompson, 1983). Free radical production is mediated through a photochemical reaction involving chlorophyll. Free radicals are potentially damaging oxidizing agents that are also known to react with metal catalysts to form the more reactive $OH^{\bullet}$ (Thain and Hickman, 2000). The loss of thylakoid membrane integrity involves perturbations on photosynthetic apparatus, thylakoid membrane fluidity and proteins (Thompson, 1988). The impact on photosynthesis involves impairment of photosynthetic electron flow which is influenced by the decline in the noncyclic electron transport (Jenkins and Woolhouse, 1981a, b).

The rate limiting nature of intersystem, photosystems I and II, electron transfer in senescing chloroplasts is brought about by a decline in the concentration of cytochrome *f*-b_6 complex (Ben-David *et al.*, 1983) and impairment of the electron transfer from plastohydroquinone to the cytochrome *f*-b_6 complex (Thompson, 1988). Chlorophyll regulates the degradation of proteins in the chlorophyll-protein complexes of the thylakoid membrane. The breakdown of chlorophyll, involving the hydroxylation reaction in chlorophyll-*a* (Maunders *et al.*, 1983), is therefore a necessary pre-requisite occurrence in the degradation of associated proteins (Thompson, 1988). It is pointed out by Roberts *et al.* (1987) that a decline occurs in the synthesis of some of the thylakoid proteins, including the α and β subunits of ATPase and the apoprotein of the light-harvesting complex. The decline in photosynthesis is only observed late in the senescence process and chlorophyll breakdown does not occur until after the decline in photosynthesis (Bidwell, 1979).

Mitochondrial function

The function of mitochondria continues until quite late in the senescence process due to the need for energy by the active processes of senescence and for maintaining homeostasis.

It has also been suggested by Bidwell (1979), Noodén (1988b) and Thompson (1988) that continued mitochondrial function is a necessary biological function required to process and transport metabolites released by senescing tissues. The continued ATP production is necessary for the plant in order to inhibit senescence while inducing necrosis. Peripheral changes involve the shut down processes of the cell's specialised functions.

Phytohormones and growth regulators

Phytohormones and growth regulators such as cytokinins and auxins influence different plant parts and function in different ways. Indole-3-acetic acid (IAA) promotes cell division, gibberellins enhance cell division in the shoot apical meristem and cytokinins stimulate cytokinesis and retard the loss of chlorophyll and proteins (Salisbury and Ross, 1985; Sabater *et al.,* 1990a). Divergent to the effects of other hormones, abscisic acid and ethylene promote senescence. The phytohormone ethylene, which is intimately connected with aging, is derived largely from methionine (Salisbury and Ross, 1985). As metabolic activities decline, methionine builds up and ethylene is synthesised (Bidwell, 1979). Ethylene is produced by all parts of seed plants including the shoot apex in seedlings (due to high amounts of IAA), nodes of dicotyledon seedlings, roots (release relatively small quantities), leaves, flowers and fruits (Salisbury and Ross, 1985). Numerous stress effects such as drought, insects, pathogenic micro-organisms, water-logging and viral attack increase ethylene production. Ethylene production in leaves increases gradually until leaves become senescent and abscise (Bidwell, 1979; Salisbury and Ross, 1985).

Phytohormones and leaf abscission

Leaf abscission has also been reported by Campbell (1993) to be a result of a change in the balance of the concentrations of the phytohormones, ethylene and auxin. Less and less auxin is produced by an aging leaf that leads to a drop in the concentration of auxin thus resulting in increased ethylene sensitivity in the abscission layer. As a consequence of the change in hormonal balance, cells in the abscission layer begin the biosynthesis of additional ethylene that inhibits the synthesis of auxins by the leaf.

Consequently, the prevailing influence of ethylene on the abscission layer, enzymes produced by abscission layer cells digest cellulose and other components of cell walls (Esau, 1965; Bidwell, 1979; Salisbury and Ross, 1985; Campbell, 1993). The synthesis of cellulases and pectinases is accompanied by a rapid rise in respiration in cells of the zone closer to the stem (Salisbury and Ross, 1985). One or more layers of the proximal cells, cells of the zone closer to the stem, increases in diameter and length while cells of the abscission zone distal to the separation zone do not.

The unequal growth of cells beside and within the abscission zone together with ongoing cell wall digestion processes cause a break between the two layers of cells (Esau, 1965; Campbell, 1993). During abscission, specific cells in the petiole differentiate to form an abscission layer between the branch or stem and the petiole in order to allow the senescent organ to separate from the branch. Pathogens do not usually invade the plant through the abscission layer due to the occurrence of a protective thick layer, known as a cicatrice, made up of suberin, wound gum and lignin over the abscission zone (Esau, 1965).

The breakdown of proteins is accompanied by a minimal increase in soluble nitrogen (Bidwell, 1979). About half of the nitrogen and phosphorus content of leaves is resorbed during senescence in order to support further growth even though calcium, which is immobile in the phloem, cannot be resorbed and re-utilised (Lambers *et al.*, 1998). Nitrogen resorption efficiency is higher in deciduous shrubs and trees than it is in evergreens.

Resorption involves several processes: the enzymatic breakdown of nitrogen- and phosphorus-containing compounds in the leaves, phloem loading and transport, and the formation of an abscission layer that breaks the transport path and causes the leaves to fall. The extent of resorption in leaves depends on the location of the leaves and the physiological status of the plant therefore, organs with strong sinks such as those located near developing leaves or new leaf growth have higher resorption efficiencies.

Stages in the breakdown of cell structure and function

Structural and functional changes preceding and occuring during senescence have been described by Bidwell (1979), Salisbury and Ross (1985), Noodén (1988b), Thompson (1988), Trippi (1990) as follows:

(i). Vacuolar protease(s) attack one or more tonoplast membrane proteins, which may be accompanied by an active synthesis of additional proteases. The closure of stomata results in this occurrence. The vacuole acts as a lysosome, secreting hydrolytic enzymes that digest unwanted cellular material.

(ii). The semipermeability of the tonoplast is weakened, thereby allowing the escape of proteases into the cytoplasm that attack the more abundant cytosol protein substrates. An increase in total proteases and lipolytic enzymes may accompany the attack on the cytosol.

(iii). In leaves, the escape of proteases from the vacuole result in a hydrolytic attack on chloroplast proteins of the envelope and thylakoids. The internal structure of chloroplasts and mitochondria are reduced prior to the enzymatic breach of their external membranes.

(iv). Sugars and amino acids, including methionine are liberated together with the production of ethylene.

(v). The increase in respirable substrates, such as amino acids, increases the respiration rate.

(vi). Soluble hydrolytic products are transported out of the affected tissue or organ.

(vii). Upon the initiation of degradative processes, synthesis functions are eliminated in organelles and cells.

(viii). Therefore senescing cells experience a substantial and lethal reduction in structure and a disruption in membraneous subcellular inclusions.

1.2.2 Mechanisms of water and mineral nutrient stress effects on physiology and growth

1.2.2.1 Mechanisms of water deficits

Water stress occurs when the matric potential of the soil reaches a threshold at which plants only continue to absorb water only as long as their water potential is more negative than that of the source of water (Baker, 1984; Taiz and Zeiger, 1998). Therefore, water stress develops as cells equilibrate with xylem potentials resulting in a loss of cell water content and a drop in cell water potential (Baker, 1984).

Cavitation, occurs following embolism, and when the air-water meniscus at the pit membrane is pulled into the functional element until a micro-bubble of air enters the element, thus interrupting the water column and decreasing xylem conductivity (Lambers *et al.*, 1998; Sparks and Black, 1999). This mainly occurs during periods of high transpiration rates when the water column in the xylem reaches a point of critical tension (Irvine *et al.,* 1998). Plants usually avoid the development of cavitation by inducing a decrease in stomatal conductance in response to ABA or a hydraulic signal from the roots.

Incidences of water stress induced cavitation are an important limitation to water transport (Sparks and Black, 1999) and if severe enough may limit growth (Lambers *et al.,* 1998). Embolism may not necessarily be disadvantageous. According to Lambers *et al.* (1998), conduits of cactus xylem cavitate under extremely high water deficit soil conditions thereby preventing the loss of water from the body of the plant into the soil. The vulnerability and variability in the occurrence and severity of the effects of xylem cavitation differ considerably among species (Lambers *et al.,* 1998). This has been confirmed by Franks *et al.* (1995) and Sparks and Black (1999) through their observations in populations of *Eucalyptus camaldulensis* (Dehnh.) and *Populus trichocarpa* (Torr. & A. Gray) respectively, from different climatic zones.

Populus fremontii and *Salix gooddingii* show complete cavitation at slightly low levels of stress whereas *Abies lasiocarpa, Acer negundo* and *Juniperus monosperma* have higher dessication-tolerant abilities (Lambers *et al.* 1998). Comparatively high water potentials have been reported by Hsiao (1973) and Richter and Wagner (1983) to lead to the full development of certain adverse effects, whereas whenever a new threshold value is reached other reactions and metabolic pathways become affected one after another. Water stress responses in a plant can also occur when a decrease in the water content of ambient air declines (Kappen *et al.,* 1994).

Osmotic adjustment and solute accumulation

A net increase in solute concentration occurs mainly in the vacuole, termed osmotic adjustment, by which the plant water potential is decreased without an accompanying turgor decrease nor changes in cell volume (Choi, 1992; Taiz and Zeiger, 1998). Osmotic adjustment allows for a slow onset of stress so that the plant has sufficient time in which to complete substantial shifts in solute synthesis and transport patterns (Hsiao *et al.,* 1976). The solute content increases during dehydration in the vacuole (Ritchie and Shula, 1984; Anderson and Helms, 1994; Johnson *et al.,* 1996) that includes organic acids, sugars and ions such as K^+. Osmotic adjustment may not be an independent and direct response to water deficit but a result of other factors such as leaf aging, decline in photoperiod and temperature.

Nevertheless, Lackso (1983) emphasises the effectiveness of osmotic adjustment as a mechanism for turgor maintenance. Osmotic adjustment has also been ascribed to the accumulation of photosynthates not yet translocated and the remainder by passive concentration as the relative water content declines. Plants have also been found to accumulate osmotically active low molecular weight compounds that include glycine betaine, proline and sugar alcohols (such as pinitol) in response to stress. An increase of pinitol in the roots of *Pinus pinaster* (L.) has been observed as a response to drought. A similar observation was made in the hypocotyl sap of *Colophospermum mopane* (Kirk ex Benth.) (Johnson *et al.,* 1996). In the same study, an increase in the root cap concentrations of pinitol, total sugars and the individual sugars, fructose, glucose and sucrose were found to occur as a response to an increase in external water stress.

The accumulation of ions in the vacuole is a protective mechanism that enables the plant to separate the ions from the enzymes in the cytosol and subcellular organelles. The compartmentation of ions facilitates the accumulation of other solutes, called compatible osmolytes, in the cytoplasm for the maintenance of within-cell water potential equilibrium (Taiz and Zeiger (1998).

Enzyme activity

In *Vigna radiata (*Wilczeck), induced water stress decreased leaf water potential which in turn resulted in a decreased activity of several enzymes that included nitrogenase, glutamine and asparagines synthase and xanthine dehydrogenase (Kaur *et al.,* 1985). Even though the production of NADH was found to decrease under severe stress, Kaur *et al.* (1985) observed a three-fold increase in the activity of glutamate dehydrogenase.

The synthesis of proteins has been reported to be sensitive to water stress, particularly in rapidly growing tissues that are actively synthesising cell wall polysaccharides and proteins (Salisbury and Ross, 1985). In the same vein, protochlorophyll formation is inhibited and the activities of enzymes such as nitrate reductase, phenylalanine ammonia lyase, among others, decrease sharply with increasing water stress. Consistent with the drop in nitrate reductase activity, nitrogen fixation and reduction also decline.

Metabolic processes

The effects of water stress on the yield threshold involve complex structural changes in the cell wall. Cell wall extensibility decreases due to stress in part because it inhibits proton transport across the plasma membrane into the cell wall, thus raising the cell wall pH. Several metabolic processes such as photosynthesis, ion transport and translocation, affected by water stress are associated with solute accumulation and maintenance (Choi, 1992). Once these metabolic processes are reduced by chronic water stress, the plant's capacity to maintain solute levels declines resulting in osmotic potential at full turgor to increase in water stressed plants.

Protein synthesis, in sugar beet leaves, was also observed by Hsiao (1970) to be affected by soil water deficit conditions. Fraction-I-proteins, and large crystal formations have been reported (Vapaavuori *et al.,* 1983) to decrease with increasing water stress in the stroma area of chloroplasts in plants subjected to stress. The decrease in fraction-I-proteins has been ascribed to an increase in proteolytic degradation.

In the drought-sensitive *Gossypium hirsutum* the total hydrolytic activity of acid phosphatase, that is the total specific activity after disruption of cellular structures by a detergent, was observed to increase in the leaf tissues of stressed plants (Vieira Da Silva, 1976). The *de novo* synthesis of enzymes or the activation of zymogen results in increased total hydrolytic activity of acid phosphatase. Under severe water stress conditions, Parkinson and Day (1983) observed the occurrence of premature senescence, a response that is also observed for the green leaf area.

Soil water deficit at the whole-plant level may have a strong impact on the development, activity, and duration of various source and sink organs (Hsiao *et al.,* 1976; Osório *et al.,* 1998). Some species exhibit changes in partitioning that favour structures involved in water uptake and transport, and increases in water use efficiency in response to water deficits (Osório *et al.,* 1998). Growth inhibition resulting from drought was reported by Kaiser (1987) and Wilson *et al* (2000) to result in sink limitation and feedback control on photosynthetic capacity.

In both herbaceous and woody plants, soil water deficits cause reductions in total dry matter, lateral branching, leaf production, and rates of leaf and shoot expansion.

Leaf expansion and photosynthesis

The inhibition of cell expansion (Hsiao *et al.,* 1976; Taiz and Zeiger, 1998; Major and Johnsen, 2001)) or cell wall stiffening (Lambers *et al.,* 1998) results in the slowing down of leaf initiation and expansion during the early developmental stages of water deficits, which leads to photosynthesis becoming more sensitive, and in indeterminate plants limits the number of leaves. This sensitivity in photosynthesis is brought about by the dehydration of mesophyll cells that results in inhibition of photosynthesis.

Another factor that influences photosynthesis during water stress is the change in Mg^{2+} concentration in chloroplasts through its role in coupling electron transport to ATP production (Taiz and Zeiger, 1998).

Dark respiration has been observed to be promoted by mild water stress even though it is decreased only at severe stress and is less sensitive to water deficits (Jones and Fanjul, 1983). Changes in respiration may affect photosynthesis even without any change in maximum photosynthetic rate and mesophyll conductance to CO_2. Since photosynthesis and the consumption of assimilates decrease in expanding leaves that are under conditions of water stress, Hsiao *et al* (1976) and Taiz and Zeiger (1998) indicate that the decrease in the amount of photosynthates exported from the leaf is an indirect effect of water stress.

Translocation is insensitive to water stress until late in the stress period, this insensitivity allows the mobilization and utilisation of reserves to where they are needed most even in conditions of severe stress. A reduction in the consumption of carbon and energy results from the inhibition of leaf expansion which also leads to the distribution of a large portion of the plant's assimilates to the root system where further growth is needed. After a substantial leaf area has been developed, plants exposed to water deficit may experience leaf senescence, abscission and finally shedding.

Stomatal aperture

Stomatal aperture and conductance are affected by a range of environmental variables that include humidity, leaf water potential, light, temperature and photon flux rates (Lösch, 1989; Ceulemans *et al.,* 1983; Kappen *et al*., 1994; Loewenstein and Pallardy, 1998; Major and Johnsen, 2001) that result in sub-optimal gas exchange capabilities in affected leaves, thereby affecting photosynthesis and respiration. The stress imposed upon plants by reduced soil water supply to the roots is transmitted to the stomata by a decrease in xylem water potential (Kappen *et* al., 1994) or by a direct ABA signal from stressed roots (Lambers *et al.,* 1998; Loewenstein and Pallardy, 1998).

The chemical signal, ABA, reported by Loewenstein and Pallardy (1998) that affects stomatal aperture may be root-originated or a combination with ABA distributed to roots from the leaves through the phloem of water stressed plants. Stomatal closure is reported by Campbell (1993) and Lösch (1993) to be affected by dehydration that may also change the turgor of guard cells, thereby modulates stomatal opening and closing. The loss of turgor results from the fact that guard cells are exposed and can lose water directly by evaporation, causing induction of hydropassive closure in stomata (Taiz and Zeiger, 1998). Another cause of hydropassive closure is the dehydration of leaves and roots which depend on metabolic processes in guard cells. The failure of guard cells to retain solutes necessary for generating the osmotic potential and turgor required for stomatal opening leads to stomatal closure (Boyer and Younis, 1983). Marked levels of stomatal closure have been reported by Salisbury and Ross (1985) to lead to the accumulation of ABA in leaf tissues. ABA tends to accumulate mainly in leaf tissues especially chloroplasts after being continuously synthesised at a low rate in mesophyll cells.

Stomatal closure and decreased chloroplast activity in membrane-associated reactions of the thylakoids limit the rate of respiration and photosynthesis, and results in thinner thylakoid lamellae in affected leaves than in leaves having a high water potential (Salisbury and Ross, 1985; Taiz and Zeiger, 1998). Once the water status of the rest of the leaf has declined, solute loss from guard cells is triggered with ABA playing an important role in the trigger of solute loss.

In photosynthesising leaves that are not subjected to water stress, the pH of the stroma is usually higher than that of the cytosol but dehydration lowers the stroma pH thereby facilitating the release of ABA (Taiz and Zeiger, 1998). Coupled to the increase in stroma pH is an increase in the pH of the apoplast resulting in net transfer of ABA from plastid to apoplast. Therefore, mild dehydration of mesophyll cells leads to the release of ABA, stored in the chloroplasts, to the apoplast that in turn is transported to the guard cells by the transpiration stream. This same condition also results in an increase in the rate of net ABA synthesis. After stomatal closure has begun, ABA synthesis increases thus enhancing and prolonging the initial closing effect of the stored ABA (Lösch, 1989; Taiz and Zeiger, 1998).

Stroma can also respond to the dehydration of some roots since during dehydration in some roots ABA is produced and exported to the xylem sap (Salisbury and Ross, 1985; Lösch, 1993; Lambers *et al.,* 1998). This root-originated ABA elicits a response in stomata as an early warning system of soil drying. Once stomatal closure occurs in the early stages of water stress, a high rate of CO_2 uptake per unit of water transpired occurs (termed water use efficiency). The increase in water use efficiency comes about due to the fact that transpiration is inhibited more than it decreases inter-cellular CO_2 concentration when stomatal closure takes place. Water use efficiency will however decline when water stress becomes severe and this will lead to an increased inhibition of mesophyll metabolism.

Abscisic acid classified as root-sourced becomes available only when a degree of leaf dehydration takes place otherwise root-originated ABA has generally been accepted as the major chemical signal from roots exposed to soil drying. In fact, Lambers *et al.* (1998) indicates that soil drying and salinity enhance the ABA concentration in the leaves that greatly inhibits leaf growth. Other factors that may cause stomatal closure independently or in combination with ABA include xylem sap pH and ion concentration.

In mature *Pinus sylvestris* (L.) exposed to conditions of water stress, stomata were observed to close sufficiently to prevent the development of substantial xylem embolism (Irvine *et al.,* 1998). Closed stomata also impacts negatively on fluorescence due to limited CO_2 availability (Schreiber *et al.,* 1994). It has also been observed by Wilson *et al* (2000) that patchy stomata closure may be involved in changing the mesophyllic CO_2 concentration under drought conditions which may in turn impact on the maximum rate of carboxylation. The opening state of stomata may depend more directly on internal parameters than total water potential, for example, hydrostatic potential or turgor, as responses to variable moisture levels seem to play major roles as parameters controlling stomatal opening in many species (Lackso, 1983; Richter and Wagner, 1983). The uptake and loss of water in guard cells changes their turgor and modulates stomatal apertures (Taiz and Zeiger, 1998). Due to the fact that guard cells are exposed to the atmosphere, they are directly affected by evaporation through which water can be lost resulting in the loss of turgor causing stomata to close.

1.2.2.2 Mechanisms of mineral nutrient deficits under conditions of water stress

Soil moisture and nutrient availability

The water status of the soil may determine the availability of mineral nutrients to the plant, other factors notwithstanding. Lowered soil water content has been assumed to interact with nutrient uptake (Kramer and Kozlowski, 1979; Ösorio *et al*., 1998) whereas water stress or reduced turgor has been known to result in a reduction in ion transport (Hsiao, 1973).

Plants growing in the field in conditions of water stress may respond to water stress in such a way that the response is more pronounced to overshadow the stress effects on ion uptake and transport within the plant. A reduction in rates of nutrient transport to the roots occurs with soil drying, in particular the amount of potassium and phosphorus is greatly reduced in the soil solution (Hsiao, 1973). The concentration of potassium and phosphorus tends to be low in dry soil because the two become fixed in the soil and the absorbing surface declines due to reduced root growth (Kramer and Kozlowski, 1979). It has been reported by Ösorio *et al* (1998) that the concentration of nitrate and phosphate in the xylem sap declines with decreasing soil water in plants that are kept fully turgid. In addition, water stress can actually aggravate nutrient deficiency under circumstances in which the nutrient is slightly deficient. Even though nutrient accumulation is reduced in conditions of water deficit, suppressed growth also has a negative impact on nutrient demand.

Water stress and mineral nutrients

Water stress effects on mineral nutrients occur in two ways: reduced transpiration and hence reduced water flow exerts an influence on ion transport, and water stress impinges upon membrane permeability and active ion transport mechanisms (Lambers *et al.,* 1998; Hsiao, 1973). Water deficit results in a change in ion uptake (in terms of potassium channels and transporters, calcium channels, ATPases), ion partitioning (tissue specific sequestration, transport and storage or excretion) and ion sequestration (cell-specific compartmentation, plasma membrane and tonoplast Na^+/H^+ antiporters) (Campbell, 1993).

Under conditions of low water availability a reduction in diffusion rates occurs in most soils because water is replaced by air in pores of dry soil (Lambers *et al.,* 1998). The result is the decrease in ion mobility which in some cases can result in reduced growth once diffusion becomes the rate-limiting process in the uptake of the most strongly limiting mineral nutrients. Therefore, in addition to declining root uptake under some conditions, low transpiration may reduce ion transport from the root to the shoot. This reduction may be affected by the compensating increase in xylem concentration at low transpiration rates.

The concentration of phosphorus in the xylem stream is increased by water stress whilst its uptake is decreased. Additionally, water stress directly impacts on the active transport mechanism and plasmalemma's passive permeability (Hsiao, 1973). A study with tomato found that the root to shoot transport of bromide, phosphate and sodium was strongly suppressed when the nutrient solution potential was lowered even though the total nutrient uptake from the solution was less affected (Hsiao, 1973). This finding confirms the fact that water stress effects on ion transport to the shoot can be considered as an independent occurrence from the corresponding stress effect on ion uptake into the root.

The nutrient regime is known to increase drought resistance in conifers. Increasing N supply to conifer seedlings has been found to reduce their drought hardiness (van den Driessche, 1992). *Pseudotsuga menziensii* (Mirb.) fertilised with N late in the season has been shown to have slightly higher water potentials than controls. N fertilisation of established trees has been shown to reduce moisture stress (Brix and Mitchell, 1986) whereas nitrogen fixation is known to be quite sensitive to water stress (Sheoran *et al.,* 1981).

In *Vigna ungniculata*, Lösch (1993) observed a decline in symbiotic nitrogen fixation with increasing water deficit under which the activity of nitrogenase declined to a fifth of its original value when nodule water potentials decreased from –0.2 Mpa to –0.48 Mpa. The decrease in nitrogenase activity occurred prior to the drop in nodule water potential and seems to be more related to the decline in soil water content than nodule water potential.

The activity of nitrogen reductase is also depressed under water stress even though increased availability of NO_3^- counteracts the reduction in reductase activity (Lösch, 1989). The concentrations of Mg^{2+} are usually 1 to 3 mM in chloroplast stroma; this concentration can increase to 9 mM and occurs during the dehydration of leaves and may be involved in the loss of activity in chloroplasts of affected leaves (Boyer and Younis, 1983).

The response of chloroplasts to high Mg^{2+} concentrations at low leaf water potentials alters photophosphorylation in *in vivo* as water potentials decrease, but the response may reflect a general chloroplast response to high ion concentrations and not specific to Mg^{2+} concentration.

1.2.3 Plant responses to water and mineral nutrient deficits

Plants are exposed to various environmental variables that may exert a disadvantageous influence on the plant either when they are below or above the optimum level required by the plant for its optimal function and growth. Stress can therefore be measured in relation to plant survival, biomass accumulation or the primary assimilation processes that are related to overall growth. Some adverse effects such as water and mineral nutrient deficits are fully developed at relatively high total water potential values whereas other biochemical reactions and pathways will be affected one after another whenever a new threshold value is reached (Hsiao, 1973; Richter and Wagner, 1983). Plants respond to adverse external factors through three mechanisms, processes of avoidance, tolerance at deficit or tolerance at excessive levels (Hsiao, 1973; Bidwell, 1979; Richter and Wagner, 1983; Seiler and Johnson, 1988; Pereira, 1994; Edwards and Dixon, 1995; Lambers *et al.,* 1998; Taiz and Zeiger, 1998).

Water stress tolerance and avoidance

When water stress occurs, plants can either avoid the stress or tolerate it under high or low water potentials. One mechanism employed in adverse environmental conditions is to slow down growth and adjust size to resources (Lackso, 1983; Pereira, 1994).

The existence of low water potentials in some species is a sign of tolerance whereas stomata closure is avoidance (Richter, 1976). In conditions of water stress, plant responses and reactions can be interpreted to be avoidance strategies whenever they help keep the stomata open despite lowered total water potentials (Richter and Wagner, 1983). When avoidance strategies fail, a plant will tolerate a reduction in photosynthesis due to water stress.

Stress tolerance can therefore be considered to be a measure of a plant's fitness to cope in an unfavourable environment (Pereira, 1994; Taiz and Zeiger, 1998). Once the level of tolerance increases as a consequence of exposure to initial stress, the plant is considered acclimatised (or hardened). In *Picea mariana* (Mill) seedlings, Edwards and Dixon (1995) found the transpiration and stomatal responses of conditioned seedlings to be superior to those of unconditioned seedlings, even though osmotic adjustment in *Thuja occidentalis* (L.) was not influenced by conditioning. Due to the foregoing, acclimatisation is distinguishable from adaptation that generally refers to a determined level of resistance genetically acquired through a process of selection over several generations (Taiz and Zeiger, 1998). This ecological fitness is more related to the efficacy of resource collection than with the efficiency of its utilization, particularly when competition is involved (Pereira, 1994).

Mechanisms of plant adaptations to water stress

Plant adaptation to stress is by changes in root/shoot ratio, growth rate and water use efficiency (Beadle *et al.,* 1993). The foregoing adjustments are often accompanied by changes in fundamental properties of water relations such as the critical water potential that induces stomatal closure, diurnal patterns of stomatal conductance and pre-dawn leaf water potentials.

The adaptive mechanisms that are developed to cope with water deficits of varying degrees may involve regulation of plant responses through changes in the plant's hormonal balance (Itai and Benzioni, 1976). Even though plants can either avoid or tolerate a level of stress, plant strategies for maintaining various biochemical processes under conditions of water or mineral stress are numerous and complex which can include physiological and morphological mechanisms.

1.2.3.1 Plant responses to water deficit

Leaf development

In conditions of water deficit, plants may respond by adjusting their leaf area (Salisbury and Ross, 1985) and number (Osiórió *et al.,* 1998) that can be considered important long-term changes that enhance the plant's fitness for a water-limited environment (Taiz and Zeiger, 1998). In apple trees, Lackso (1983) found that rapid leaf adjustment was a drought escape or avoidance mechanism for water stressed trees. Plant leaf area adjustment results from the inhibition of cell expansion, in particular the reduction of cell wall synthesis (Salisbury and Ross, 1985), which slows leaf expansion early in the development of water deficit in order for the plant to transpire less water. The limiting of transpiration through reduced leaf area can lead to the build up of heat unless another process offsets the lack of cooling, such as the dissipation of extra energy as sensible heat loss. Plants can also respond to the build up of heat by altering the interception of radiation through wilting which changes the angle of the leaf (Taiz and Zeiger, 1998).

It has been indicated by Lackso (1983) that leaf morphological adaptations to water deficit may involve folding about the midrib, thereby reducing radiation absorption by the exposed leaves and raising leaf water potentials. Once plants are water stressed after the development of a substantial leaf area, the leaves will senesce and abscise (Salisbury and Ross, 1985). Plant water deficit, through its negative effects on leaf expansion and leaf senescence, has a corresponding negative impact on biomass accumulation (Beadle *et al*., 1993; Lösch, 1993).

To maintain photosynthetic productivity for the whole season, plants have developed mechanisms for avoiding drought that requires a leaf area that develops early in the season to have a very slow physiological aging rate (Lackso, 1983). In the desert drought deciduous shrub, *Encelia farinosa* Gray, seasonal changes in leaf pubescence occurs based on prevailing mesic or drier conditions (Ehleringer, 1982). The changes bring about dramatic modifications in leaf spectral characteristics that significantly alter leaf absorbance from 29% under driest conditions to 81% under mesic conditions.

Therefore, these changes result in lowered leaf temperature and rates of water loss. Decreased rates of transpiration have also been observed in *Pinus taeda* (L.) subjected to a severe water stress regime (Seiler and Johnson, 1988).

Root growth

Root growth and distribution follow the water reserves of the soil (Lackso, 1983) as the soil water declines and the soil dries, the soil-root contact decreases due to a shrinkage of both the soil and roots, thereby resulting in a reduction in water uptake which in turn leads to changes in stomatal closure, and a decrease in photosynthesis and biomass production (Beadle *et al.*, 1993). The growth of roots is affected more by water stress than shoot growth (Hsiao, 1973) which is supported by findings of (Seiler and Johnson, 1988) in *Pinus taeda* (L.). Hsiao (1973) also indicated that root extension growth is reduced at the onset of osmotic stress. However, Itai and Benzioni (1976), Lösch (1993), Johnson *et al.* (1996) and Lambers *et al.* (1998) found water depletion in the localised root environment to promote root growth in order for the plant to exploit further soil water reserves. Hence with decreasing water availability root growth is enhanced at the cost of aboveground biomass accumulation.

This is supported by Rood *et al* (2000) who observed the promotion of root elongation and a retardation of shoot growth due to water stress effects brought by moderate rates of water table decline that produced an overall reallocation of growth processes. The accumulated ABA inhibits shoot growth that conserves water use but root growth appears to be promoted, thereby increasing water supply (Salisbury and Ross, 1985). Therefore, the higher restriction on shoot growth (Choi, 1992) results in the increase of the root/shoot ratio (Itai and Benzioni, 1976). In the Seiler and Johnson (1988) study, root dry weight exhibited a higher decrease of 71% compared to the 63% for the shoot dry weight in seedlings exposed to severe water stress conditions.

The inhibition of leaf area expansion more than root elongation results in the leaf area ratio and leaf mass ratio decreasing whereas the root mass ratio increases as a response to increasing water stress (Lambers *et al.*, 1998). However, the root-shoot relationship is governed by a functional balance between water uptake by roots and photosynthesis by the shoot (Taiz and Zeiger, 1998).

The functional balance is shifted when water supply decreases by distributing a greater proportion of the plant's assimilates to the root system where they can support further growth. This results from the inhibition of leaf expansion that reduces the consumption of carbon and energy. Therefore, in conditions of soil water deficit deep rooting into wet soils becomes a second line of defence against drought.

Photosynthesis and plant growth

The growth of *Pinus sylvestris* (L.) was observed to decline in the year after a severe water deficit had occurred, indicating that the water deficit resulted in reduced assimilation in the year of drought (Irvine *et al.,* 1998). A similar observation was made by Wilson *et al* (2000) that assimilation rates frequently decline under drought which is evidence of drought-induced reductions in photosynthetic capacity. In plants in which changes in leaf spectral characteristics take place with changing soil moisture status, such as *Encelia farinosa* Gray, high leaf pubescence reduces leaf temperature and water loss but results in optimal photosynthetic rates that are necessary for optimum growth (Ehleringer, 1982).

In severely stressed plants, a decline of 10% or less in total energy dissipation through photosynthetic carbon dioxide fixation has also been reported (Björkman and Demmig-Adams, 1994). In *Pinus taeda* (L.) it was found that photosynthetic rates acclimatise to low plant water potentials in seedlings subjected to water-stress conditioning (Seiler and Johnson, 1988), most likely as a process of drought tolerance even though Ceulemans *et al.* (1983), in *Hevea brasiliensis* clones, and Björkman and Demmig-Adams (1994) observed a sharp decline in net photosynthesis with increasing water stress. Experiments in willow (*Salix* sp. 'aquatica gigantea') revealed the disappearance of large crystal formations, Fraction-I-proteins, with increasing water stress under weak light conditions (Vapaavuori *et al.*, 1983).

Even though low irradiance may be the primary stress factor for crystallisation during the growth period of willow, the increase in proteolytic degradation of Fraction-I-proteins was brought about by the introduction of water stress. The increase in protein degradation caused a disappearance of the crystals, decrease in the rate of CO_2 uptake and subsequently photosynthesis declined.

1.2.3.2 Physiological effects of mineral nutrient deficiencies

Plant growth over different regions of the earth is limited by the availability of nutrients, particularly nitrogen and phosphorus (Woolhouse, 1983). Almost all the essential elements required for plant growth are involved in some aspect of chlorophyll metabolism. Therefore, it becomes inevitable that a large part of stresses brought about by nutrient deficiency will have substantial effects on photosynthesis and plant growth. However, it is difficult to ascertain the linkage between the deficiency of a particular mineral element and the observed effect due to the complex nature of the interactions between mineral elements (Kramer and Kozlowski, 1979).

Mineral nutrients are categorized based on function, by Kramer and Kozlowski (1979) and Taiz and Zeiger (1998), into the following broad groups

(i). Constituents of plant tissues, such as calcium in cell walls, magnesium in the chlorophyll molecule, phosphorus in phospholipids and nucleoproteins and nitrogen and sulphur in proteins

(ii). Copper, iron and zinc are prosthetic groups or coenzymes of some enzyme systems

(iii). Activators or inhibitors of enzyme systems such as manganese and magnesium

Mineral nutrient deficiencies depend on the functional role and mobility of the particular nutrient in the plant (Campbell, 1993). Deficiency symptoms of nutrients that are mobile will be apparent first in older plant organs due to the preferential allocation of nutrients, which are in short supply, to young and growing tissues. However, deficiency symptoms of nutrients that are relatively immobile in the plant will first appear in young and growing tissues due to the fact that nutrients already in older tissues are retained because of their relative immobility. According to Kramer and Kozlowski (1979) and Campbell (1993), the commonest nutrient deficiencies are those associated with the levels of nitrogen, phosphorus and potassium.

The reduction in allocation of assimilates to new leaf area rather than the rate of photosynthesis per leaf area that results in decreased growth was found to be related to reduced nitrogen supply (Pereira, 1994). Also, low levels of nitrogen lead to a reduction in leaf expansion (Lambers *et al.,* 1998), the final leaf size as well as the release of axillary buds and the accumulation of starch (Kramer and Kozlowski, 1979; Schulze *et al.,* 1991). Additionally, Salisbury and Ross (1985) indicated that drought and nitrogen deficiency promote leaf loss by hastening senescence. The effect of nitrogen on biomass allocation is similar to that of phosphate probably through the effects of phosphate on nitrogen. It has been reported by Lösch (1993) and Björkman and Demmig-Adams (1994), that nitrogen deficits seriously increase stress effects such as the decrease in intrinsic photosynthesis rate and leaf resistance. The reduction in growth observed by Ösorio *et al* (1998) has been ascribed to the interference of reduced nitrogen concentration on growth regulators such as cytokinin without changes in the nitrogen concentrations in leaves in water deficit conditions. Such changes in the concentrations of nitrogen and sulphur are due to emendations in the synthesis, structure and function of coenzymes, amino acids, proteins, nucleic acids and other chemical compounds.

Mineral deficiencies have been observed to decrease carbohydrate synthesis and their translocation to growing plant tissues (Kramer and Kozlowski, 1979). Apart from reducing photosynthesis and increasing respiration, potassium deficiency has also been found to inhibit the translocation of carbohydrates that is mainly pronounced in boron deficient trees with phloem necrosis (Kramer and Kozlowski, 1979). Plants allocate relatively less biomass to leaves and more to their roots when nitrogen or phosphate are in short supply, therefore plants invest in plant parts that acquires the limiting nutrient at the expense of the part that has a requirement for the limiting mineral element (Lambers *et al.,* 1998). The process of mineral nutrient may involve the release of hormones by organs or tissues acting as nutrient sinks, to other organs that cause the release of some of their nutrients (Salisbury and Ross, 1985). Plants experiencing adverse conditions, which may be followed by organ senescence, degrade large molecules (except for cell wall constituents) into smaller, more readily translocatable structures through which nutrients are conserved by being deposited in other plant parts (Salisbury and Ross,1985). A similar process takes place in leaves in which nutrient salvage by remobilisation occurs prior to leaf abscission.

CHAPTER 2

2. SEEDLING GROWTH AND ASSOCIATED EFFECTS OF PHENOLOGICAL CHANGES IN *Pterocarpus angolensis*

2.1 SEASONAL VARIATIONS IN SHOOT AND ROOT BIOMASS ASSOCIATED WITH LEAF PHENOLOGY OF *Pterocarpus angolensis* SEEDLINGS

2.1.1 Introduction

Pterocarpus angolensis grows in a natural habitat that is extremely diverse in altitude, rainfall, soil type and temperature. It is found as far north as Kiwele (7° 46' S, 35° 41' E) in Tanzania and south to Nelspruit (25° 30' S, 30° 58' E) in South Africa. The altitude ranges from 1300 m above sea level at Kasama (10° 13' S, 31° 12' E) to 570 m above sea level at Kande (11° 56' S, 34° 06' E). The species occurs in areas with a high variability in mean annual rainfall (about 1400 mm in Kasama to less than 850 mm in Singalamwe, 17° 41' S, 23° 23' E) and temperature (14° C to 30° C) as well as soil type; from dystrophic soils of the Kalahari sands to the sandy-clayey soils that extend to Solwezi (12° 11' S, 26° 25' E) and Kasama. In such an altitudinally and climatically variable habitat, the phenotypic characteristics of the species are expected to show high variability relative to the specific ecosystems in which it is found. This trend has been observed in a high rainfall Miombo (*Brachystegia, Isoberlinia* and *Julbernadia)* forest in Solwezi in relation to a low rainfall Zambezi teak forest in Machile (17° 26' S, 25° 02' E). The major differences have been in relation to low stocking and smaller crowns in Solwezi which is a high rainfall and semi-closed woodland, compared to relatively high stocking and large crowns in the Kalahari sands of south-western Zambia and north-eastern Namibia which has low rainfall and a more open woodland compared to Solwezi. The only commonality amongst the diverse ecosystems is the unimodal rainfall pattern that influences and confines regeneration, germination and re-sprouting of dormant seedlings, to November and February just prior to and within the summer rainy season.

Naturally regenerated seedlings accumulate biomass between November and March during the rainy season and prior to substantive declines in the water table. The quantity of biomass in seedlings is a result of the difference between the production process of photosynthesis and consumption through respiration and mortality. Consumption processes of harvest and herbivory are added to consumption only when estimating the biomass in a forest (Brown *et al.*, 1999), but rarely under plant growing programmes such as glasshouse experiments in which harvest and herbivory are almost absent, unless harvest takes place as part of the experiment. Changes observed in the biomass of seedlings can be a result of the silvicultural regimes applied, pests and diseases as well as changes in climate. The major climatic change that significantly impacts on *P. angolensis* seedlings is the winter season during which the shoot dies back under field (Boaler, 1966; Vermeulen, 1990) and glasshouse (Kasumu, 1998; Munthali, 1999) conditions. It is feasible to infer that the total biomass of seedlings in which shoot die-back occurs declines in relation to the time preceding and after the season of shoot die-back.

The experiment was carried out in order to ascertain changes in biomass allocation in the root and shoot, associated with phenophases (Table 2.1.1), and to describe the origin of new shoots after shoot die-back has taken place.

Table 2.1.1. Description of phenophases observed in *Pterocarpus angolensis* seedlings

MONTH	PHENOPHASE
March - May	Late maximal leaf expansion and onset of leaf yellowing
May - July	Continued leaf yellowing and shoot die-back – the leaves and stem gradually die
September - November	Leaf flush and early leaf expansion
December - March	Maximal leaf expansion

It was therefore hypothesised that biomass remains constant in each phenological phases.

2.1.2 Materials and methods

P. angolensis seed that was used in this experiment was obtained from the accessions listed in Table 2.1.2.

Table 2.1.2. *Pterocarpus angolensis* seed sources used in the study

COUNTRY	LOCATION	LATITUDE AND LONGITUDE	COMMENT
Malawi	Kande	11° 56' S, 34° 06' E	
Malawi	Mbongolo	11° 03' S, 33° 52' E	Accessions were bulked
Malawi	Ngara	10° 15' S, 34° 06' E	
Malawi	Phalombe	15° 50' S, 35° 40' E	
Malawi	Skull Rock	14° 25' S, 35° 28' E	
Namibia	Hamoye	17° 56' S, 20° 01' E	
Zambia	Kalulushi	12° 50' S, 28° 03' E	Kalulushi and
Zambia	Mansa	11° 12' S 28° 53' E	Mansa were bulked

A single seed was sown per 1.2 *l* black plastic container that had a depth of 30 cm, in December 2000 in the Department of Forest Science glasshouse (33° 56' S, 18° 52' E) of the University of Stellenbosch. Seed pre-treatment involved nicking of the seed by removing a part of the seed coat (Kasumu, 1998; Munthali, 1999).

The potting mixture was made up of sandy-loam soil made up of 2 parts sandy soil mixed with one part of fine compost, with a pH (KCl) of 6.2 (appendix Table 2.1.1). The potting mixture was inoculated with soil containing mycorrhiza from a *P. angolensis* site in Nelspruit.

The experimental design was

Replications = 6

Number of seed sources = 3 (Malawi, Namibia, Zambia)

Number of seedlings per seed source = 210

Number of seedlings per replication = 105

Plot size = 35 seedlings

Sampling = 5 seedlings per seed source per replication in each of the four phenophases.

The phenophases corresponded to seedling ages of 3 (March 2001), 6 (June 2001), 9 (September 2001) and 12 months (December 2001).

The study was conducted in a covered glasshouse with a uniform water regime but with varying temperature (Fig. 2.1.1), light and humidity conditions that were influenced by the external environment. Environmental control in the glasshouse was limited to cooling by two thermostatically controlled exhaust fans that were set at 35° C. The roofing for the glasshouse is made of fibre carbon glass that reduced solar radiation by 30%. Seasonal and diurnal ranges of solar radiation varied based on the seasonal and diurnal patterns obtained in the Western Cape.

Water was applied in the morning and cool hours of the late afternoon at about 70 ml per plant during the hot summer season. The water regime was scaled down to 35 ml per plant every 48 hours during the winter season. The scaled down water regime was necessitated by the dormancy phase of the species that occurs during winter as well as reduced evapo-transpiration rates.

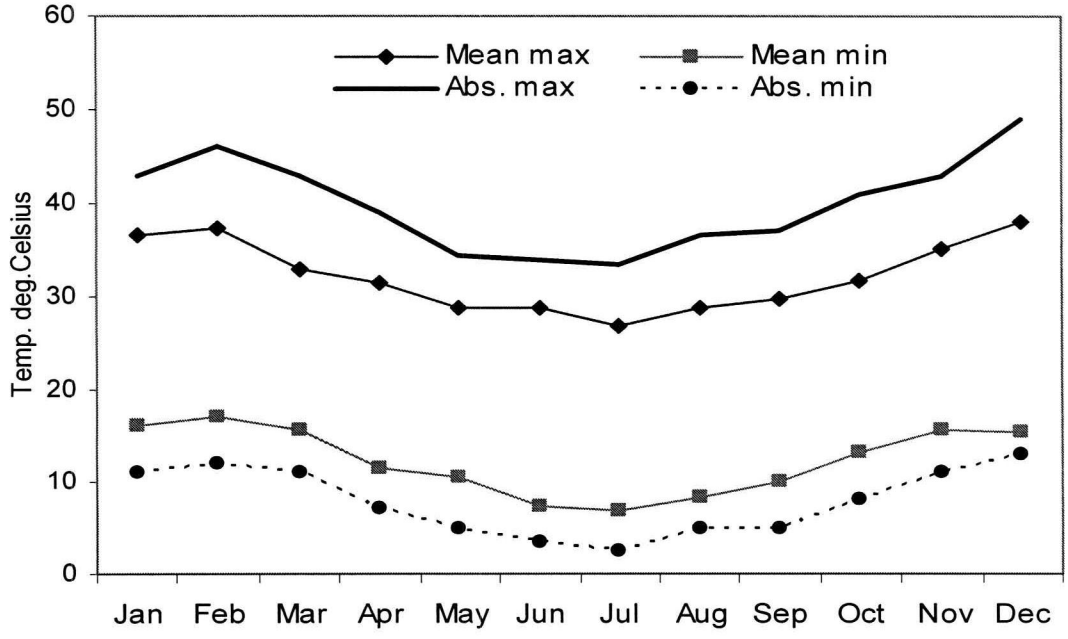

Figure 2.1.1. Mean and absolute monthly temperatures in the Department of Forest Science nursery of the University of Stellenbosch. Mean max=mean maximum temperature, Mean min=mean minimum temperature, abs. max=absolute maximum temperature, and abs. min=absolute minimum temperature. Mean maximum and minimum monthly temperatures are means of maximum and minimum day temperature for each month. Absolute maximum and minimum temperatures are the actual maximum and minimum temperature for each month. Temperature was recorded from 2001 to 2003.

A supplement as an additional source of nitrogen, ammonium sulphate, containing 21% N [210 g/kg N (w/w)] was applied to each seedling at 10 ml of a 2 g/*l* solution on a weekly basis in the first season of growth. Thereafter the application was reduced to one application per month. Iron chelates (with 6% Fe) were mixed with water at 400 mg/*l* and applied at 1 *l*/10 m^2 of glasshouse space. The iron foliar spray was applied very lightly as chemical scorching occurred when the solution left a coating of iron on the leaf after drying. Improvement after iron application was observed within two weeks. Additionally seedlings were raised on a commercial fertilizer, Multifeed® which was applied as a foliar application. In all foliar applications a wetting agent was mixed with the nutrient solution at 1 ml/5*l*. Nutrient supplements were applied based on the fact that *P. angolensis* seedlings, in an earlier experiment, were found to exhibit nitrogen and iron deficiency symptoms.

Non-nutritional glasshouse problems with the seedlings occurred with respect to aphids, mites and red spiders which were dealt with by the application of Metasystox R® at 2 ml/*l* for aphids and mites, and cyhexatin 600 SC® at 1 ml/2*l* for red spiders.

At each phenophase (90) seedlings were separated into stem and root which were cleaned and oven dried at 80^0 C for 24 hours. Seedlings sampled in December 2001 were oven dried at the same temperature but for 48 hours due to the fact that plant samples did not dry up sufficiently when dried for 24 hours. Root collar diameter, i.e. 1 cm below the top, was measured using an electronic diameter calliper. Shoot height and root length were measured with a 30 cm ruler prior to oven drying whereas dry weight was determined after oven drying.

The linear model for the analysis was $Y_{ijk}=\mu+\beta_i+\gamma_j+\lambda_k+\varepsilon_{ijk}$, where Y_{ijk} is the response obtained at the i^{th} block, j^{th} seed source and in the k^{th} phenophase, μ is the mean of the population, β_i is an effect due to the i^{th} block ($i = 6$), γ_j is an effect due to the j^{th} seed source ($j = 3$), λ_k indicates an effect due to the k^{th} phenophase ($k = 4$) whereas ε_{ijk} is a random error term associated with main effects. The ε_{ijk} were assumed to be normally distributed with mean 0 and unknown variance, and independent.

Relationships between shoot height and seedling age, root length and seedling age as well as root length and shoot height were tested by the examination of the linear model $Y= a + \beta x + \varepsilon$ in which the response variable, (Y), represented shoot height and root length whereas the predictor variable, (x), represented seedling age and shoot height. The model $Y = a + \beta x + \beta x^2 + \varepsilon$, was tested for root biomass, shoot biomass, total biomass yield and the ratio of shoot to root biomass yield against seedling age as well as for the relationship between shoot and root biomass.

The response variable (Y) represented biomass yield and the ratio of yields whereas the predictor variable (x) represented the seedling age and root biomass. Shoot dry weight data was transformed by applying a square root function, $y = \sqrt{x}$ (Ott, 1993) whereas shoot height data was not transformed. The remaining variables were transformed using

$y = \sqrt{(x+1)}$

where

y = transformed data and

x = untransformed data (Snedecor and Cochran, 1989).

Data transformation was carried out due to the fact that tests of normality proved that the data was not normally distributed. Additionally, the time of bud break, leaf expansion and yellowing as well as shoot die-back were noted.

The experiment was not taken to its final end due to declining numbers of seedlings for each treatment because of mortality. Therefore only the four phenophases of the first year occurring in March, June, September and December 2001 were assessed and analysed. The phenophases corresponded to the season for the transition between leaf maximal expansion and yellowing, shoot die-back, leaf flush and leaf expansion.

An experiment to assess tap-root extension growth for 10 months was carried out and is described in appendix Table 2.1.4. The experiment was necessitated by the rapid growth of seedlings grown in the 30 cm deep containers.

2.1.3 Results and discussion

The results from this experiment are presented graphically and in tabular form. Qualitative observations such as the time of bud break, leaf flush and the origin of new shoots are discussed together with results from quantitative data. Pictures are used to illustrate the qualitative aspects of the study. The objective and results from this experiment do not provide an explanation as to why seedlings died. The mechanism of mortality and survival in *P. angolensis* remains an uncertain event and need further exploration.

The discussion arising from the evaluation of relationships (appendix Table 2.1.3) include both significant and non-significant relationships. The raw data and descriptive statistics of a brief observation study of longitudinal taproot growth in 2.4 m long tubes are tabulated in appendix Table 2.1.4 to illustrate the influence of soil depth on the longitudinal growth of the taproot.

2.1.3.1 Shoot height and root length growth

The shoot and root structures of *P. angolensis* exhibit different rates of growth in each of the four leaf phenophases. Shoot height significantly differed amongst the four seasons (appendix Table 2.1.2) and was found to decline from the peak of the growing season towards the shoot die-back season (Fig. 2.1.2) partly due to the occurrence of shoot die-back as well as increasing mortality. Shoot height, and not root length, was also observed to be significantly different amongst the three seed sources.

The lowest mean shoot height was observed between shoot die-back and leaf flush due to the fact that the new growing season's shoots had not yet been fully regenerated. The foregoing is supported by the 2% increase in mean shoot height between September and December that is largely due to the onset of shoot growth. Additionally, leaf turnover is a continuous process between leaf flush and the end of expansion that lasts from September to March.

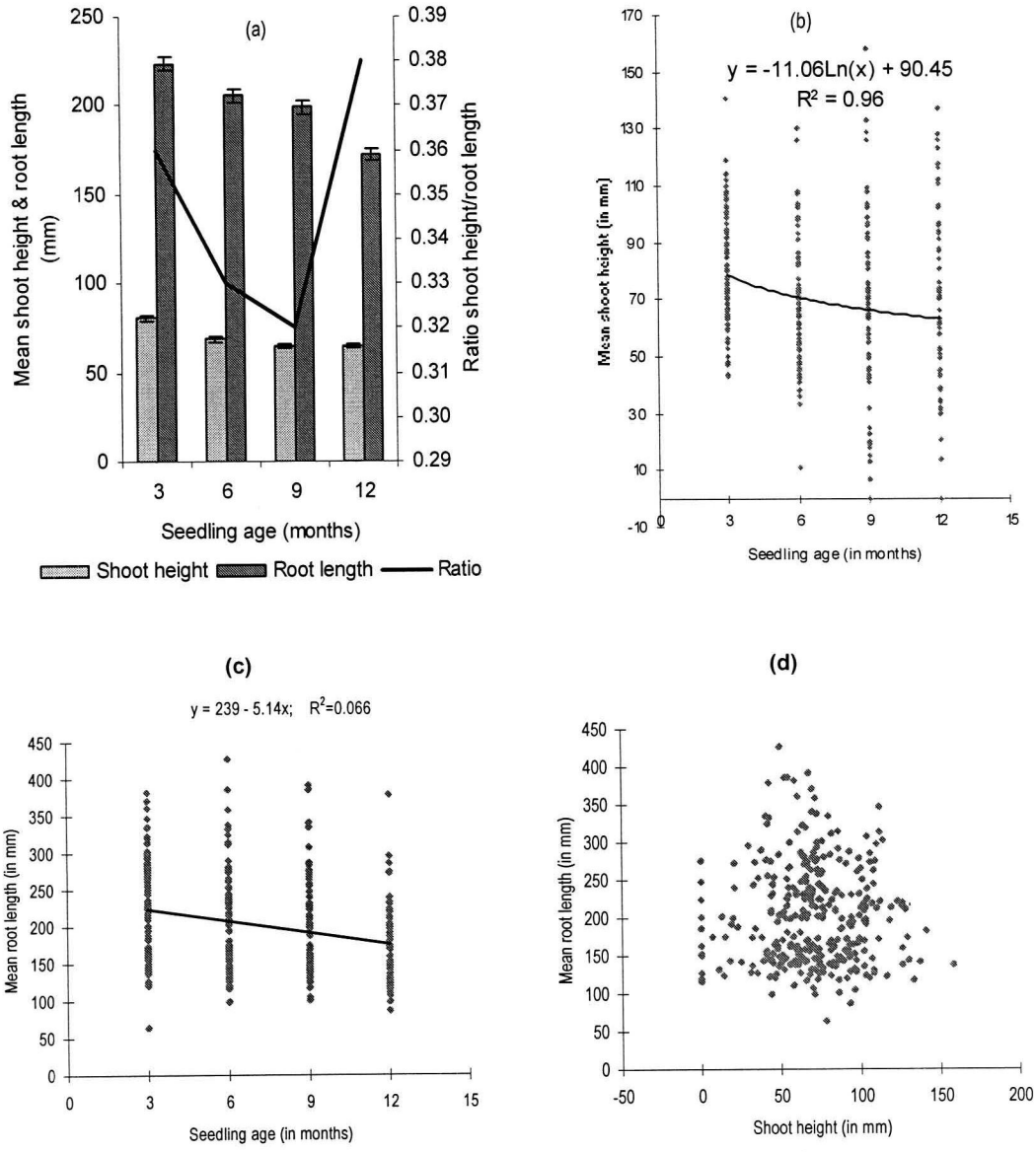

Figure 2.1.2. (a) Seasonal and age related changes in shoot height and taproot length and the ratio of shoot and taproot length against the age of seedlings; (b) relationship between mean shoot height and seedling age, (c)linear relationship between mean taproot length and seedling age and (d) relationship between taproot length and shoot height. Bars on each series in (a) represent standard errors.

The current season's stem gradually senesces during shoot die-back after which another shoot (or shoots) is regenerated either from adventitious buds located on the top of the root, axillary buds on the unsenesced lower portion of the shoot or sides of the whole shoot (Fig. 2.1.3). The latter case only occurs in exceptional situations where senescence of the whole shoot does not occur and is only characterised by the complete shedding of leaves. Therefore, since height measurements only took place in live shoots, heights of older senesced shoots were not taken into account.

Shoot growth was observed to be high during leaf flush. Seedlings that did not undergo complete or partial shoot die-back rarely accumulated as much shoot height, once a new shoot was regenerated, compared to shoots that undergo complete or partial shoot die-back.

Partial die-back involving over 50% of the shoot stimulates a shoot growth similar to that occurring in seedlings that experienced complete die-back. The only difference is in the number of newly regenerated shoots which may be higher in partial than complete die-back depending on the number of dormant buds on the unsenesced portion of the stem.

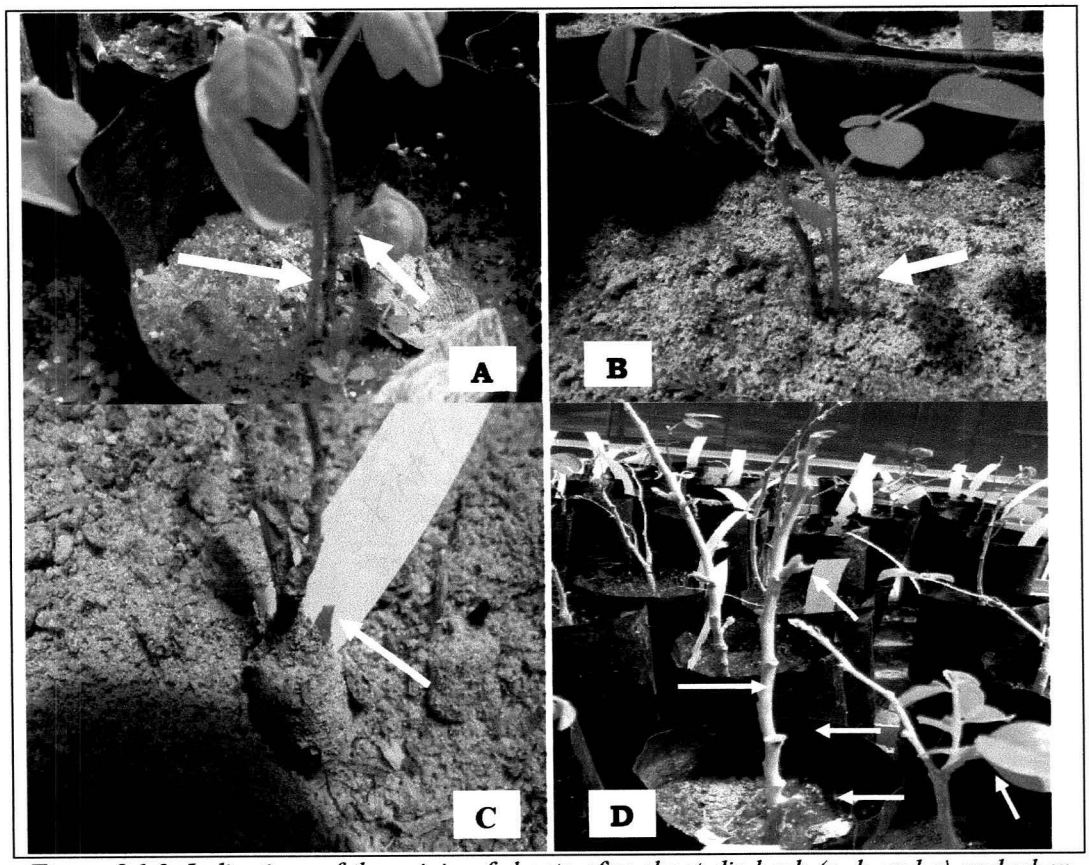

*Figure 2.1.3. Indications of the origin of shoots after shoot die-back (a, b and c) and when shoot die-back does not take place or is incomplete (d). In (a) and (b) new shoots regenerate from the base of the senesced shoot as well as the side of the seedling (refer to arrows). The shoot from the base of the original shoot is very vigorous compared to the shoot developing on the previous season's shoot. New growth in (c) originates just below the soil surface and root collar on what is apparently the top of the tuber. In (d) seedlings that had not gone through shoot die-back regenerate shoots from dormant axillary buds on most of the stem except the top 5 cm which had gone through some form of tissue degeneration during the previous winter. Shoot regeneration sometimes takes place next to the dividing line between the fresh part of the shoot and the degenerated portion. (**Scale:** 1: 3).*

The previous season's shoot does not grow as much as the newly regenerated shoot due to the fact that the shoot senescence process is arrested either after all the leaves are shed or before the youngest leaves are shed. In either case, the shoot develops an unhealthy yellowish green coloration that is indicative of partial loss of cellular integrity and functional ability. Assuming that this process is irreversible it is impossible for the plant to restore the lost cell viability that will enable it to put on shoot height, but due to the plant's evolved ability to regenerate new organs, a shoot is produced that will render the older shoot redundant.

Seedlings with unhealthy old shoots occasionally have multiple shoots that gradually assume the same colour as the older shoot, finally shrivelling and dying off. Frequent aphid attacks are common in sickly shoots. A similar trend was observed in the Mooifontein (25° 24' S, 3° 41' E) and Sudwala (25° 23' S, 3° 41' E) caves areas in Nelspruit in which naturally regenerated *P. angolensis* seedlings growing in Mooifontein, which is fire free showed poor growth and small leaves of a light green colour compared to seedlings that were growing in Sudwala, which experiences annual bush fires. Seedlings in Sudwala appeared to be healthy with large lush green leaves.

Even though the ages of these seedlings were not known, the differences in plant size, leaf size and colour were apparent. Leaf colour in *P. angolensis* seedlings is a key indicator of health and state of development. Dark green leaves are indicative of healthy and vigorously growing seedlings whereas light to pale and yellowish leaves are signs of either nutrient deficiencies, particularly iron, incomplete shoot die-back or the onset of leaf loss. In each case, the season of occurrence of each of the mentioned factors is important in determining the probable cause with the exception of incomplete shoot die-back whose effects may be long lasting.

The commonly observed terminal-bud-scale scars that separate two growing seasons were not conspicuous in this species. Terminal-bud-scale scars of the previous growing season are not as visible as those of the most recent season. The most recent season's growth can only be detected by the proximity of leaf scars in a part of the stem with shorter than average internodes.

Stem or twig growth of the current year sets off from the previous year's growth left by a ring of terminal-bud-scale scars that are very proximal to the shoot apex. In *P. angolensis* the previous season's growth may disappear with shoot die-back. In situations where the shoot does not die-back, thus resulting in the failure of continued shoot growth, no terminal-bud-scale scars will separate two growing seasons due to the fact that the apical meristem that has overwintered rarely continues to be meristematic as it was in the previous season. Significant numbers of bud scales during the dormancy phase are apparent and bud scales are easily visible with the naked eye. The previous season's stem that did not die-back will either be unhealthy or very slow growing in the subsequent season.

Therefore, if stem growth is thereafter taken over by a newly regenerated shoot, the previous season's stem may gradually lose its viability and is shed off. In most cases plants with such stems do not grow well. Field implications for Figure 2.1.3c are that *P. angolensis* wildlings growing in areas with an abundant organic matter layer or seedlings whose tuber is not buried deep enough below the surface, may be affected severely by fire in the dry season or by drought which may influence shoot regeneration in the growing season.

The effect of fire on subsequent survival may also be negative for seedlings that have not completed their shoot die-back process since the remaining fresh shoot may be severely damaged thus complicating the physiological adjustment to the effect of fire. Therefore, the development and final stage of shoot die-back may have serious regeneration implications under field conditions. Secondly, surviving roots of seedlings might have to be covered by at least 2-5 cm of soil to avoid either detection by burrowing animals or scorching by fire. This may enhance the survival and subsequent development of seedlings. The depth mentioned earlier may also be an evolutionary characteristic that may have an important developmental relationship with seasonal rainfall. Probably roots buried closer to the soil surface detect climatic changes or the onset of rains much earlier that signal the onset of growth.

Even though it was difficult to accurately determine root length, particularly beyond three months of seedling growth, the estimates obtained indicated significant variations (appendix Table 2.1.2) in root length over time in which a relative decline in root length from the third month of growth was observed (Fig. 2.1.2a). This is explained by the branching habit of the taproot that makes it difficult to clearly identify the main root in plants in which it occurs.

The significant variations in shoot to root length ratio (appendix Table 2.1.2) are apparent in its decline from the third to the ninth month due to the fact that root growth in *P. angolensis* is not in direct proportionality to shoot growth (Fig. 2.1.2c) because of the intervening phenomenon of shoot die-back. In a particular seedling, one root will generate several generations of shoots depending on the evolutionary frequency of shoot die-back phases in any particular habitat. The resumption of shoot growth during the leaf flush season, September to December, leads to an increase in the shoot height to root length ratio.

The impact of decreased seedling growth during shoot die-back also affects the shoot to root dry weight ratio which exhibits a similar trend to that of shoot height to root length ratio. Effects of shoot die-back are long term in some seedlings and yet in others shoot die-back acts as a stimulus for accelerated growth and development during the growing season. Completely different functional and developmental relationships between shoot height growth and root length increase may be obtained after the seedling has graduated out of the suffrutex phase.

Sub-optimal temperatures that are experienced during the Western Cape winters as well as low than average temperatures that occur in natural *P. angolensis* habitats probably result in injury, dehydration or plant organ dysfunction by affecting normal cellular function.

In chilling-sensitive plants, translocation out of the leaf when the leaf is chilled to 10°C can be inhibited. Temperatures below 10°C are common in the Western Cape, which may result in negative physiological effects in *P. angolensis* that are similar to what is obtained in chilling-sensitive plants.

The main phenological traits of component genera of the Zambezian region, in which *P. angolensis* occurs, are greatly influenced by temperature (Menaut *et al.*, 1995). Most chemical reactions as well as enzyme-catalysed reactions are temperature sensitive (Pereira, 1994; Taiz and Zeiger, 1998). In *Pinus sylvestris* (L.), low temperatures negatively affect source-sink relationships, thereby changing the seasonal dynamics of non-structural carbohydrates (Oleksyn et al., 2000). Temperature has a significant effect on photosynthesis (Pereira, 1994), but some plant species are capable of positive net CO_2 assimilation at temperatures below and above optimum (Taiz and Zeiger, 1998).

Other processes affected by temperature include apoplastic phloem loading, germination, osmotic adjustment, respiration and vernalization. Osmotic adjustment may not only be an independent and direct response to temperature but a result of other factors such as leaf aging, decline in photoperiod and water deficit. The adjustment allows for a slow onset of stress so that the plant has sufficient time in which to complete substantial shifts in solute synthesis and transport patterns (Hsiao *et al.*, 1976), which include organic acids, sugars and ions such as K^+.

Assuming that osmotic adjustment and other physiological processes are arrested at an advanced stage due to a decline in mean daily temperatures, the ability of the seedling to regenerate shoots may suffer from the residual effects of the extensive shifts in solute synthesis and transport patterns already in place. This may partly explain why year old plants, that have partially senesced shoots, do not exhibit vigorous shoot growth during leaf flush.

In cases where the shoot is used as a variable for describing seedling development, shoot height and biomass of *P. angolensis* seedlings still remain the most appropriate variables to describe seedling development due to the fact that root collar diameters may be misleading in situations where the stem is partially or completely dead. In such stems chances are high that an adventitious bud will arise from the top of the root to constitute the viable shoot, thus complicating the interpretation of root collar data so obtained.

Limited applications of this observation applies in time terms when the seedling is still in its suffrutex stage due to the fact that it may have no shoot for close to 5 months of the year during which developmental assessments based on the shoot may be irrelevant. Therefore in assessments involving the shoot in different seasons of growth a *spatial summation* may apply in which one either obtains the full or no response from the shoot as a response variable for seedling growth assessment.

2.1.3.2 Root tuber development in *P. angolensis* seedlings

The development of a tuber or 'carrot-like root' in *P. angolensis* root systems is rapid and is assumed to be influenced by the size of the container, in terms of depth and volume, in which the seedling is growing. The early indications of tuber development can be observed in the earliest stages of root growth (Fig. 2.1.4a), in the first month after germination. After three months of growth, the carrot like structure in the root is very noticeable and keeps on increasing with age. This is illustrated by the nine month old seedling in Figure 2.1.4b that clearly shows the "carrot-like" root structure.

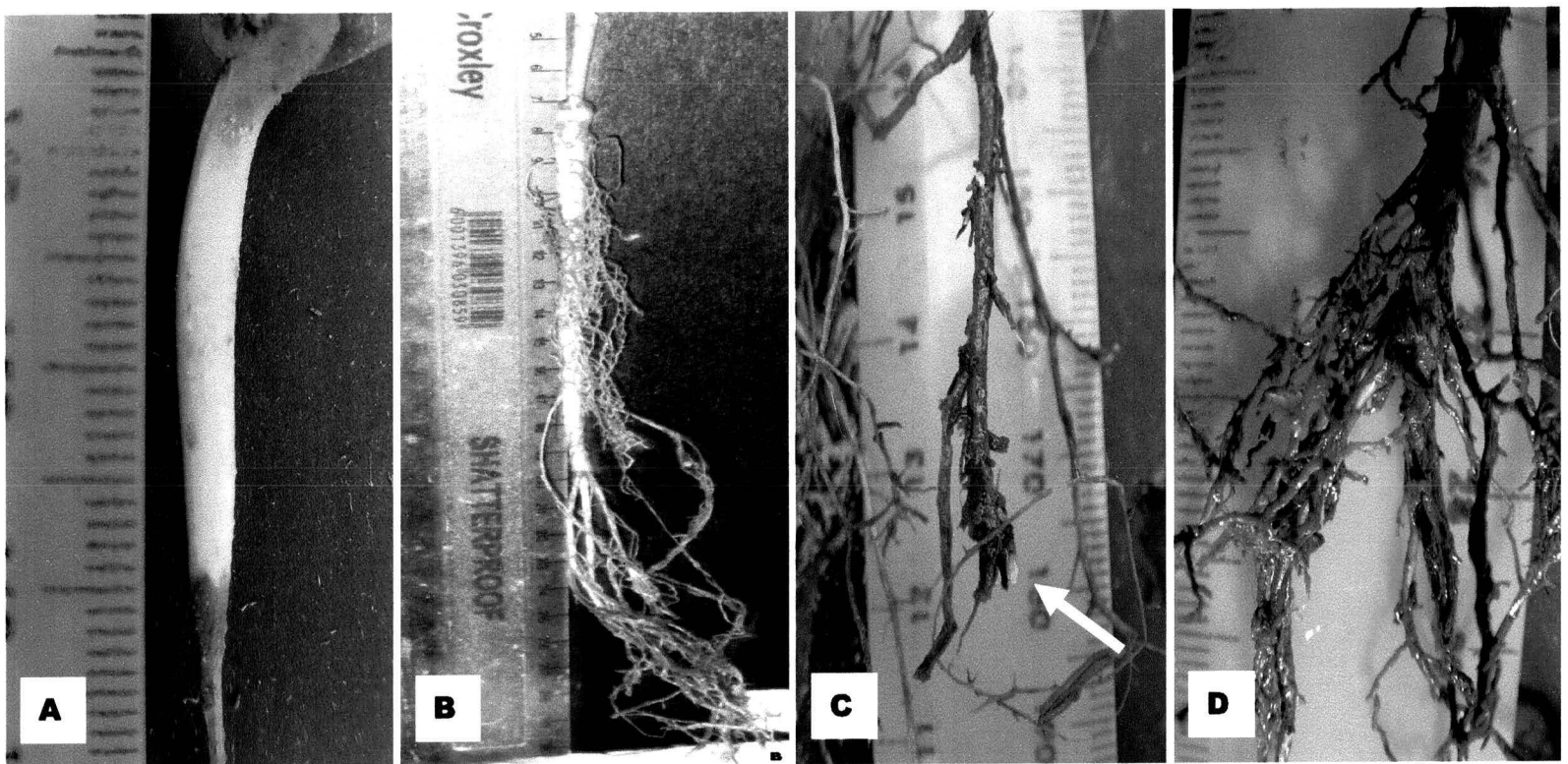

Figure 2.1.4. Changes in root tuber structure with age in P. angolensis seedlings. In (a) a one month old seedling exhibits root tuber structure that is apparent in the white or upper part of the root whereas this is amplified by a nine month old seedling (b) in which the carrot like root structure is apparent. The lower and dark coloured portion of (a) constitutes the rapidly extending part of the root. Branching and thickening (c and d) towards the tips of the taproot is ascribed to excessive water levels at the base of sealed containers or in combination with secondary growth from lateral meristems. The arrow in (c) points out the root apical meristem which has a colour very different from the rest of the root.

In this experiment root collar diameters, which significantly varied from season to season, ranged from a mean of 5 mm after 3 months growth to 7 mm in 12 months (Table 2.1.3). The roots of seedlings grown in 30 cm deep containers were observed to grow to a maximum mean depth of 22.3 cm with mean top and mid root diameters of 7 mm and 5 mm, respectively, within 12 months.

Table 2.1.3. Means of the root length, diameter and dry weight of *P. angolensis* glasshouse seedlings

Season	Root length (mm)	Root collar diameter (mm)	Middle of root diameter (mm)	Root dry weight (g)
Leaf flush	199^{b}(±6.6)*	6.1^{b}(±0.13)	4.46^{a}(±0.14)	0.74^{a}(±0.04)
Leaf expansion	173^{c}(±6.4)	6.6^{a}(±0.17)	4.05^{b}(±0.17)	0.72^{a}(±0.05)
Maximal leaf expansion and leaf yellowing	223^{a}(±7.1)	5.2^{c}(±0.09)	3.46^{c}(±0.11)	0.37^{b}(±0.02)
Shoot die-back	206^{ab}(±7.3)	5.5^{c}(±0.18)	3.00^{d}(±0.13)	0.71^{a}(±0.05)

**/:Figures in parentheses are standard errors for each mean*
Root trait means followed by the same superscript are not significantly different

The decline in root length (Table 2.1.3) is partly a manifestation of the loss of positive geotropism in the taproot. Apparently this loss may have a positive effect on the formation of the tuber since plant resources saved from allocation to taproot extensibility are redirected to root volumetric growth, which is reflected in the increase in root dry weight. It was expected that the root length would exceed 300 mm by coiling at the bottom of the container but the root of *P. angolensis* being positively geotropic and partly sensitive to water logging, loses its unitary root character when the taproot is restricted, by becoming branched and the branches thickening excessively (Fig. 2.1.4).

The abandoned vertical taproot extension rarely resulted in a proliferation of long and clearly identifiable primary lateral roots. The branches that were observed resembled primary laterals even though these were thicker in comparison to the usual primary lateral roots. Secondly, the branches broke easily upon being handled, and appeared "dead".

If excessive branching and thickening is the way that *P. angolensis* responds to water logging, then taproot extensibility may significantly be affected when the taproot encounters a high water table or excessive water levels occur. Ordinarily, the root apical meristems continue supplying cells for the root to grow in length since roots do not show determinate growth, particularly in young seedlings of perennials. The thickening of taproot endings may have been caused by water logging alone or in combination with secondary growth from lateral meristems. The softness of the tissues of the thickened taproot lengths is probably due to the fact that lateral meristems replace the epidermis with a secondary dermal tissue that thickens but rarely hardens due to the absence of added layers of vascular tissue. This view is supported by the discovery of very few taproots with root tips that can be classified as root apical meristems, instead the tips of the branches are generally the same colour as the rest of the thickened taproot lengths which appear to be brown, dead tissue. In taproots with apical meristems, the taproot tip is creamy in colour and transluscent and appears conspicuously alive (Fig. 2.1.4d).

Container effects on the development of the taproot of *P. angolensis* were apparent when the taproot characteristics from this experiment and an observation experiment were compared. The description of the observation experiment is in appendix Table 2.1.4. At ten months old, seedlings grown in plastic tubes measuring 240 cm long with a diameter of 8.5 cm had a mean taproot length of 61.6 cm and longer primary laterals, with a mean length of 17.2 cm (appendix Table 2.1.4). Roots growing in long tubes attained mean top and mid root diameters of 5 mm and 0.4 mm with only a noticeable "carrot-like" root in the first 10 months. A striking feature of the roots from the long tubes is the poor formation of the tuber which is much more apparent in the shorter containers (30 cm deep). The common feature in shorter containers of taproot branching at tips is absent in all plants that were grown in long tubes, necessitating the view that the presence of a blockage to taproot extension results in branching and the early expansion of the taproot into a large but short tuber.

The poor formation of the tuber, in deeper containers, is an indication of a uniform allocation of biomass in the root system that is important in maintaining the active taproot depth necessary for the continued access of the plant to moisture in the soil.

In the root apical meristem of seedlings grown in shallow containers, an inhibition probably occurs in the frequency of longitudinal cell divisions that contribute to root extension growth, but radial cell growth is stimulated in the meristematic cells along the length of the root starting from near the root collar and moving downwards.

The carrot-like structure that is prominent in the short container seedlings might not only be a reaction to restricted soil depth but may also be "an intelligent" phytomass structural reprogramming to increase its sink strength particularly for storage of assimilates. Therefore, the loss in length is compensated for by the gain in volume. Longer laterals reflect the absence of root restrictions to extension and also reveal the natural growth habit of the *P. angolensis* root system in covering as much ground as possible to facilitate an effective mineral nutrient and water accumulation system.

The implication of the longer taproots from long tubes may be a revelation of field growth characteristics of the species when it grows in ecosystems with deep and unrestricted soil environments. Seedlings growing in shallow soils may not accumulate a lot of taproot length but instead may put on a lot of shoot growth that "obscure" the deficiency in taproot length. Even though the taproots from long tubes are much longer than those from short tubes, it was evident that the stem height was the exact opposite. Since taproot extension growth was observed to be accompanied by poor tuber formation, it may be assumed that the plant allocates more reserves to the development of a longer taproot than it makes available for volumetric growth. Subsequently, the "saving" made from inhibited taproot extensibility is reasonably allocated to the increase of the photosynthetic surface as well as stem growth. Longer and deeper taproots are necessary for accessing more moist soil levels in order to facilitate the availability of water and mineral nutrients to the root during the long dry season that occurs in the natural habitat of the species.

2.1.3.3 Shoot and root biomass allocation

Shoot and root biomass, in terms of oven-dry mass accumulation, significantly varied (appendix Table 2.1.1) from one phenological phase to the other (Fig. 2.1.5a). Patterns differed between the shoot and root biomass.

Shoot biomass declined during the season of shoot die-back but increased during the leaf flush or growing season whereas mean root biomass showed a slight decrease between September and December and a steady increase between March and September. The slight drop in the increase of mean root biomass accumulation between June and September was due to the adverse effect of shoot die-back. The linear trends (Fig. 2.1.5b to e) illustrate the shoot and root biomass changes over the assessment period. Total seedling biomass accumulation was not significant (appendix Table 2.1.1) due to the fact that decreases in shoot biomass accumulation during shoot die-back are compensated for by low increases in root biomass accumulation (Fig. 2.1.5a). Shoot and the total biomass also significantly varied amongst the seed sources.

The fact that root biomass increase is more likely to lead to an increase in shoot biomass concurs well with the postulation that the root of *P. angolensis* has a higher influence over the shoot as a sink that facilitates increased biomass accumulation from stored reserves of assimilates. Therefore, the increase in root biomass has a physiologically beneficial effect on the growth and development of the shoot since large roots may reflect a high storage volume for energy reserves which are used for shoot growth during the early days of spring growth prior to the full development of an adequate photosynthetic surface.

Since the suffrutex phase in this species refers to the stage in its biological life history during which a seedling does not grow above the shrub layer, which is a direct reference to shoot height, taller or larger seedlings that grow above the shrub layer do so probably due to the fact that they have a sufficiently large root tuber that stores sufficient reserves to enable the developing shoot to attain maximum height above the shrub layer. The increase in root biomass between June and September can be explained by the fact that the root uses stored photosynthetates to accumulate biomass. A general view of growth from stored reserves, for all seedlings, is not possible when considering the fact that not all seedlings in the glasshouse experience shoot die-back. The degrees of shoot die-back greatly vary and this may lead to an assumption that a reduced level of photosynthesis still occurs in winter which may explain the increase in shoot biomass, unless seedlings use stored reserves contained in the root for the purpose of shoot biomass accumulation.

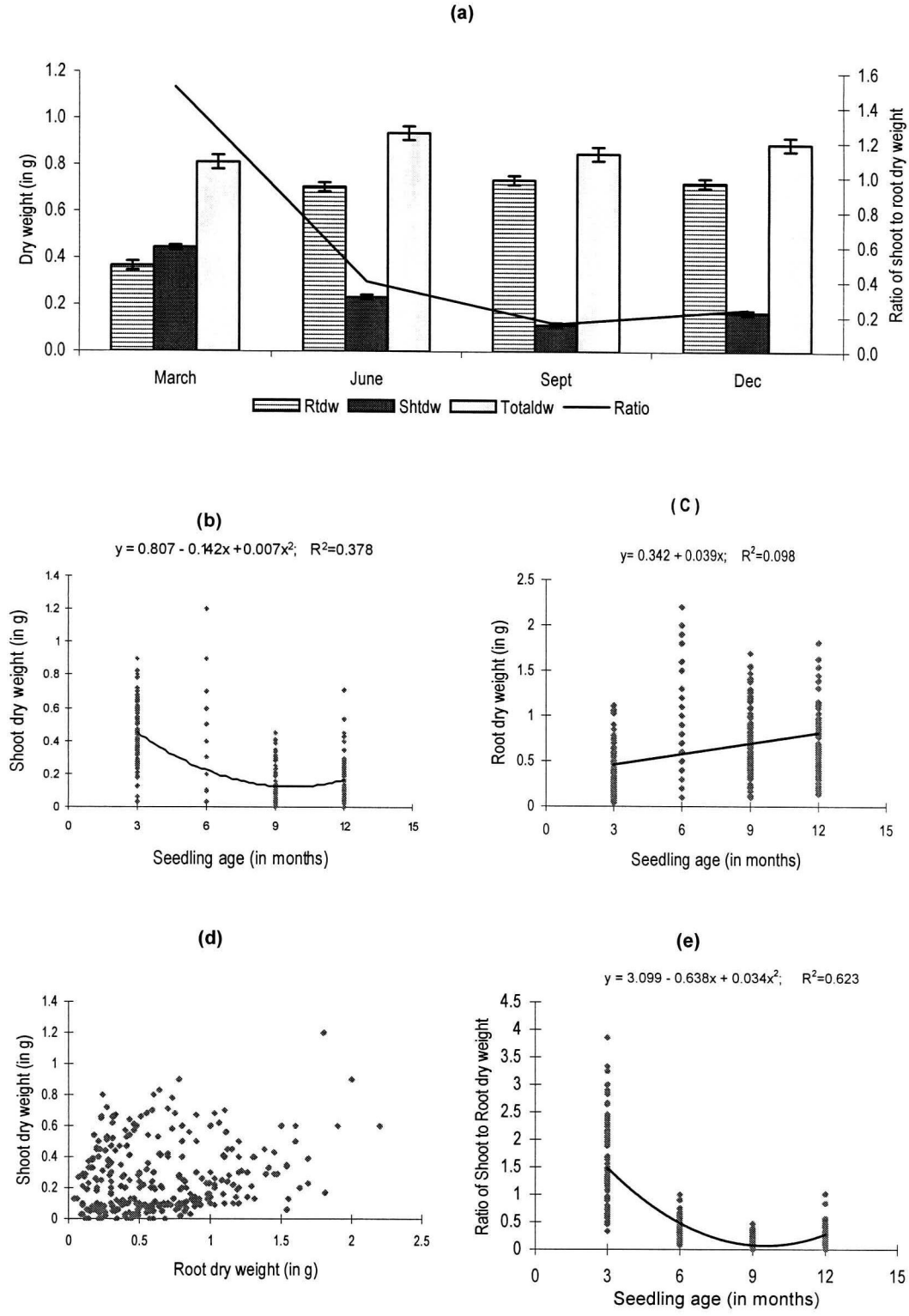

Figure 2.1.5. Mean root and shoot dry weight as well as the ratio of shoot to root dry weight in P. angolensis seedlings (a). Relationships between shoot biomass and seedling age (b), root biomass and seedling age (c), shoot and root biomass (d) as well as the ratio of shoot biomass to root biomass and seedling age (e). Captions on each bar of (a) indicate the standard error. (Rtdw = root dry weight, Shtdw = shoot dry weight; Totaldw = total dry weight and ratio = ratio of shoot to root dry weight). Bars on top of each series represent the standard error for that series. Months are used on the x-axis in (a) instead of the names of seasons in order to optimise space.

The shoot to root dry weight ratio, obtained in the 1.2 *l* containers, was found to be significant with the exception of the means of ages nine and twelve months which were not significant. Root dry weight decreased to about 10% of its March value in September before rising again by December. The high shoot to root dry weight ratio obtained by March reflects the rapid shoot growth that is associated with *P. angolensis* seedlings which is reflected also in the mean shoot height. Increases in the ratio of shoot to root biomass in the period between September and December signal the onset of the growth season and regeneration of shoots. The shoot also becomes woody much earlier than the root due to the fact that the root takes time to build up its cellulose content. The rapid shoot growth is an evolutionary adaptation that enables the plant to develop a large photosynthetic surface in the first few days after germination.

The increase in root growth surpasses shoot height growth from the time that leaves have reached full expansion and can adequately avail root growth of the sufficient photosynthetates for growth and storage. The foregoing is supported by the decline and increase in shoot and root biomass, respectively, between March and September.

Increased biomass in the root that lowers the shoot to root ratio may also be related to the increased development of storage tissue in preparation for adverse conditions, in this case decreased moisture and temperature, and the loss of or decrease in the photosynthetic surface. The quality of the remaining photosynthetic surface is significantly poorer due to excessive chlorosis and poor leaf growth and development for seedlings that produced leaves late in the growing season. Specifically, seedlings experiencing shoot die-back had slightly higher mean root collar diameters (6.3 mm) than seedlings that had stems or shoots throughout the year (5.7 mm), whereas the mean root biomass, 0.42 g, in seedlings with senesced shoots was lower compared to the mean root biomass, 0.63 g, of seedlings not experiencing shoot die-back. The latter difference may be explained by continued biomass accumulation late in the season in seedlings not experiencing die-back. The low mean root biomass obtained in seedlings with senesced shoots may be due to the fact that the lack of shoots during the shoot die-back and leaf flush season did not avail the root with the requisite photosynthetates for biomass accumulation. Root length of seedlings experiencing and not experiencing shoot die-back exhibited a trend similar to that of root biomass.

In Figure 2.1.3 (c) and (d), it is obvious that the number of apical and lateral shoots that are produced by each plant are different and directly influence the size of the photosynthetic surface in the early days of growth. In rare cases, stems that had experienced die-back were observed to remain in the same state as far as December even though this was not observed to occur between December and June. The time of occurrence of shoot die-back in the Western Cape varies based on the severity and length of the winter season. This observation may also be reasonably extended to the occurrence of shoot die-back under field conditions in relation to the rainy season and the tropical winter months.

2.1.3.4 Relationship between above and below ground organs

The significant linear relationship existing between shoot height and root biomass as well as root collar diameter and root biomass (appendix Table 2.1.3) may be useful in estimating the below ground biomass from shoot height. In situations where shoot die-back has occurred, it may be possible to use the root collar diameter in order to have an estimate of the below ground plant part. This relationship also reveals the fact that root collar diameter and biomass are closely related for both seedlings experiencing shoot die-back and those not experiencing shoot die-back (Fig. 2.1.6).

In both relationships, taller shoots and roots with large root collar diameters are indicative of a correspondingly higher root biomass than in seedlings with comparatively shorter shoots and narrow root collars. Probably the rapid root extensibility and continued biomass accumulation is not only for the purpose of accessing deep laying moist soil zones but is also for the development of storage and mechanical support tissues necessary for plant development. Storage parenchyma, composed of cells with thin walls of cellulose (Harder *et al.*, 1967; Weier *et al.*, 1974), collenchyma and sclerenchyma (Mauseth, 1988) are developed by the plant during the rapid root volume growth for the storage of starch, in the former, which the root has access to during the winter months. Most likely around the same time during shoot growth and to facilitate temporary storage, assimilatory and transfusion parenchyma as well as aerenchyma are developed in the shoot tissues.

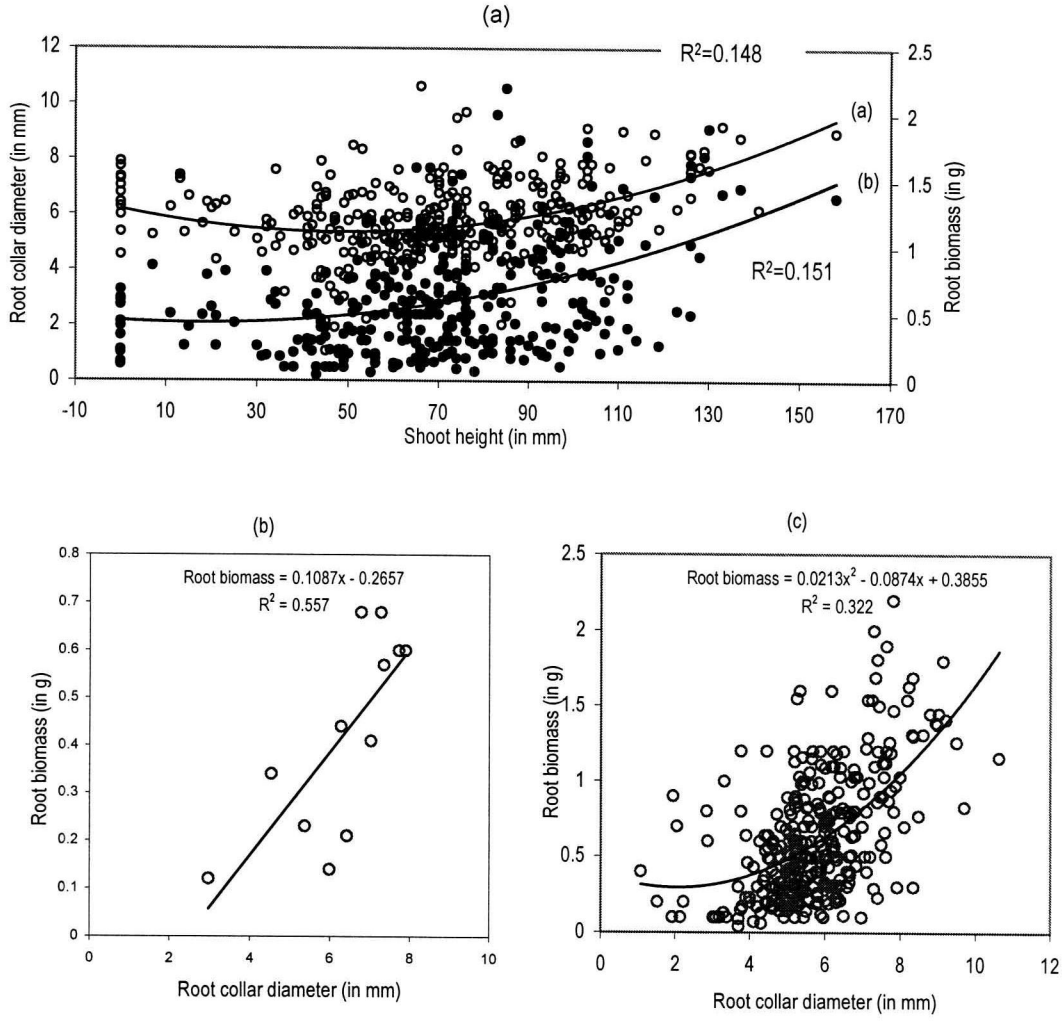

Figure 2.1.6.Linear relationships amongst shoot height, root collar diameter, and shoot and root biomass. (a) shoot height of P. angolensis seedlings plotted against (○) root collar diameter [(a) shows $y=6.015-0.029x+0.0003x^2$] and (•) root dry weight [(b) shows $y=0.492-0.0030x+0.00006x^2$]. In (b) root collar diameter of seedlings without stems (seedlings that experienced shoot die-back) is plotted against root biomass and in (c) root collar diameter of seedlings not experiencing shoot die-back is used to predict root biomass.

If these processes are complementary, roots develop these tissues based on a corresponding development of functionally complementary tissues in the stem and leaves. Therefore, the sink strength of a particular root volume, assuming die-back had taken place, may act as a stimulus and determinant of shoot size. In shoots that do not exhibit discontinuities in growth, it is difficult to make the same assumption partly due to the fact that such shoots rarely show the healthy and vigorous growth observed in shoots that experienced die-back.

Shoot height is strongly related to the diameter of the root collar in *P. angolensis*, indicating that roots with higher diameters are more likely to regenerate taller shoots (appendix Table 2.1.1). This is partly due to the significant positive correlation between root biomass and root collar diameter (Fig. 2.1.6). Due to the carrot-like structure of the taproot, a volumetric increase of the root has a corresponding impact on root collar diameter increase hence the direct effect on taproot structure. The assumption can therefore be made that under a similar set of conditions, probably even under field conditions, quantitative data obtained for shoot height and root collar diameters may be used to draw conclusions as concerning the likely magnitude of root biomass and root collar.

2.1.4 Conclusion

The physiological effects surrounding shoot die-back, which occurs in winter, are mainly apparent through declined shoot growth and biomass accumulation. Changes in the growth patterns observed in phenophases, are indicators of changes in biomass occurring between leaf flush in the shoot and throughout the year in the root. To what extent these are influenced by the seed sources is not known at present. Phenophases such as leaf loss and shoot senescence, whether shoot die-back occurs as a complete or partial event, are directly related to the decline in above-ground biomass and declined increase in the root. Shoot die-back has a minimal negative impact on root biomass accumulation compared to the shoot itself but phenophases such as leaf flush, expansion and growth are directly related to increased biomass accumulation. Even though the leaf phenophases may be taken as physiological circles of preparation for shoot die-back, not all shoots that shed leaves experience shoot die-back. Leaf shedding in *P. angolensis* is part of the deciduous nature of the species and not necessarily a part of stem senescence. Whether a seedling sheds or does not shed leaves before die-back occurs, an incomplete process of shoot die-back has long lasting negative effects on the growth and development of the whole plant which usually results in stunted and sickly seedlings.

Depending on the type of shoot die-back, sequences of shoot die-back not only delay incremental height gain but negatively affect stem straightness. Poor stem form is very apparent in situations of incomplete or partial stem die-back due to the fact that a newly regenerated lateral shoot, which becomes the dominant and main shoot, only suppresses the growth of the previous season's shoot. The shoot that had over-wintered remains part and parcel of the seedling rendering the stem to be crooked. Only in situations where there is complete stem die-back will one obtain a straight stem, as well as in situations where there is no stem die-back and the terminal apical meristem continues to be meristematic in the subsequent season. This may explain why some young naturally regenerated seedlings of *P. angolensis* have a bushy appearance, usually without a dominant shoot.

The most important observation is the continuous increase in root biomass that occurs throughout the year regardless of the occurrence of shoot die-back. Assuming the continuous root biomass increase to be an evolved characteristic, it might be possible that continuous root biomass accumulation is obtained under field conditions albeit at a reduced scale given the harsh conditions in the species' natural habitat. If the longer taproot length observed in long tubes is an illustration of field growth characteristics of the species, phytomass increment is possible in seedlings that are not restricted in taproot extensibility. The strong relationship between shoot and root phytomass is an indication of the fact that the development of a sufficiently large root tuber is an important developmental profile of *P. angolensis* that guarantees a larger shoot in the subsequent growth season. This means that the current shoot rarely benefits from the current season's root increment but only the next shoot generation will take advantage of the pre-shoot die-back root increment, the dividing line in shoot generations being the season of shoot die-back.

Observations during this glasshouse study indicate that the term "shoot die-back" is generally used to refer to stem die-back. Since not all plants experience shoot die-back under glasshouse conditions and the fact that *P. angolensis* is deciduous, i.e. sheds leaves during the dry and cool season, it is appropriate and reasonable to refer to this phenomenon as *stem die-back* which appropriately and reasonably refers to the part of the plant that experiences die-back and also excludes the foliage which is part of the shoot.

The foregoing is supported by the fact that whether a plant does or does not experience *stem die-back* it is more than likely to shed leaves by the fact that it is a deciduous species. Whether or not artificial supplementation of soil nutrients can increase root biomass accumulation is beyond the scope of this study, its usefulness is not certain either, unless enhanced biomass accumulation is physiologically beneficial to the immediate post-die-back and subsequent seedling development. Since it has been proven that root biomass accumulation does not cease during shoot die-back, it may be beneficial for long term seedling development to effect fertilizer application directly to the soil during the period of leaf yellowing and shedding as well as shoot die-back.

The resulting effect may be increased or accelerated root biomass accumulation that may result in pronounced increases in the size of the shoot and reduced frequencies of shoot die-back. Whether or not this increase in root biomass during winter affects the levels of stored reserves that are required for the initial growth and development of a sufficiently large shoot is not known.

It may be pointed out that the mechanism of nutrient uptake from the soil is correlated to the shoot and therefore chances of enhanced nutrient uptake by a "shootless" root system might be significantly minimal unless the nutrient uptake dynamics of this species are different due to continued root growth in the absence of a shoot.

2.2 ANATOMICAL CHARACTERISTICS OF THE SHOOT APICAL MERISTEM RELATED TO THE PHENOLOGY OF *Pterocarpus angolensis* DC. SEEDLINGS

2.2.1 Introduction

Pterocarpus angolensis DC., a member of the subfamily *Papilionoideae* of the family *Leguminosae*, occurs in southern Africa including Tanzania, (Boaler, 1966; Vermeulen, 1990). The species is considered endangered due to poor stewardship and long-term adverse climatic conditions (Munyanziza and Oldeman, 1995; Musokonyi, 1998 and Stahle *et al.*, 1999). Problems related to stewardship include inappropriate inventory procedures (Stahle *et al.*, 1999), unknown natural regeneration dynamics, non-existent and inappropriate silvicultural technology (Munyanziza and Oldeman, 1995) and forest management methods specific to ecosystems in which the species occurs. Natural regeneration of *P. angolensis* in the Miombo (*Brachystegia, Julbernadia, Isoberlinia*) woodlands is greatly affected by the long dry season, annual fires and in some areas by browsing (Munyanziza and Oldeman, 1995; Stahle *et al.*, 1999). *P. angolensis* is sensitive to overshadowing, therefore high natural regeneration rates are mainly in open woodlands or open areas of closed forests, particularly in high rainfall areas. Grazing and browsing have been widely assumed to impact negatively on the species ability to regenerate. A condition termed the *suffrutex* (Boaler, 1966; Vermeulen, 1990) in which the shoot dies back to about 3 cm below ground level during the cool part of the dry season has been reported and assumed to be the major hindrance to successful regeneration and ageing of *P. angolensis*.

In its natural habitat, the phenology of the seedlings is categorised into four major plant growth cycles: (1) bud break followed by leaf flush; (2) leaf growth and development to leaf maturation; (3) leaf yellowing and shedding and (4) a progressive degeneration and death starting with apex senescence. This is called shoot die-back, and implies the death of the last season's above ground growth.

The shoot apical meristem (SAM) of angiosperms is divided into two regions, the tunica and the corpus based on cell alignment and division (Esau, 1965; Fahn, 1969; Clark, 1997; Haecker and Laux, 2001). The structure of the apical meristem may be flat or form a mound that has been estimated to have a diameter of 100 to 300μm (Taiz and Zeiger, 1998). Cells of the apical meristem, that directly descend from embryonic cells, are relatively small and isodiametric with thin cell walls comprising mainly protopectins and very little cellulose (Harder *et al.*, 1967; Taiz and Zeiger, 1998; Tasaka, 2001). Densely packed cells in the core and mantle have relatively large nuclei filled with dense cytoplasm, lacking large central vacuoles. The tunica consists of one (monostratose) or more layers (multistratose) of cells (Mauseth, 1988; Sussex, 1989; Kerstetter and Hake, 1997) and has a duplex nature (Lyndon, 1998; Taiz and Zeiger, 1998) consisting of two cell layers. The number of layers in the tunica, characteristic of the vegetative stage, may change during ontogeny or transition to the reproductive phase (Esau, 1965; Harder *et al.*, 1967; Lyndon, 1998). The configuration of the corpus, positioned immediately below the tunica cell layers, may vary between stratified and nonstratified (Esau, 1965; Lyndon, 1998; Taiz and Zeiger, 1998).

The meristematic cell initiating zones or promeristems are subtended by two derivative zones where organogenesis and histogenesis start. Even though promeristems are the primary source of meristematic cells, the size and shape particularly of the tunica changes between successive plastochrons and seasonal growth (Esau, 1965; Mauseth, 1988; Lyndon, 1998). The shoot apex fluctuates between maximal and minimal sizes in relation to when the next primordium is about to be initiated or a new primordium has just been initiated, respectively (Harder *et al.*, 1967; Lyndon, 1998). Variations in shape and structure of the shoot apex, during the interval between the initiation of two successive leaf primordia, depends on the level at which a leaf primordia is initiated (Esau, 1965). Recent studies have also indicated that lateral outgrowths within SAM do not occur when auxin transport, either towards or away from a primordium, is inhibited (Reinhardt *et al.*, 2000; Berleth and Sachs, 2001). In angiosperms, leaf initiation has a relatively consistent pattern though the depth of the tunica is variable.

The duration of plastochronic changes as well as the size and shape of the SAM are greatly influenced by environmental conditions and the stage of plant development that also influence the relative size and shape of the apex (Lyndon, 1998). Changes in size and shape of SAM do not only occur with cycles of leaf and stem formation but with seasonal growth as well (Taiz and Zeiger, 1998). Seasonal growth has a greater impact on the size and structure of the shoot apex. The onset of dormancy is accompanied by secession of cell division in the apical meristem that may lead to the disappearance of the cytohistological zones leaving only the tunica-corpus organisation (Taiz and Zeiger, 1998). Amongst environmental variables impacting negatively on plant growth and function is temperature which has been observed to lengthen the cell cycle in vegetative apical domes in *Silene* plants grown at 13°C (Lyndon, 1998). The cell cycle was observed to be 57 hours compared to 26 hours in seedlings grown at 20°C, a higher temperature of 27°C produced a longer cell cycle of 93 hours. The controls on apical growth rate and cell cycle are not known particularly for species that grow slowly under favourable environments. Since the rate of increase of cell volume controls the growth rate, dimunition of the cell cycle length which is also determined by the rate of increase in cell volume, is indicative of declined growth rate (Noodén, 1988a; Lyndon, 1998).

The apical dome has been shown to enlarge with age and this has been observed to be the case in *Picea* in which the relative growth rate of the apex increased during the first 3 months after sowing (Lyndon, 1998). In Cactaceae, Mauseth (1988) observed that adult plants have larger SAM than seedlings, whereas in *Pereskia* and *Rhipsalis* the meristems enlarge relatively little with the age of the plant. The adult apex was found to be 3000 times as large as that of the seedling of *Homalocephala* and *Echinocactus*.

Meristem growth patterns are governed by genetic and hormonal influences (Mizukami, 2001; Sussex and Kerk, 2001 Yuceer *et al.*, 2002) apart from environment cues and age. The classification of apical structures should reflect fundamental structural differences to facilitate a better understanding of the behaviour of meristems.

The study was aimed at ascertaining changes in the anatomical features of the shoot apex, from longitudinal sections, that are associated with phenological changes (phenophases) in order to determine whether anatomical changes are associated with the occurrence of shoot die-back. The hypothesis was made that morphometric properties and cell number in the apical dome of the shoot apex do not change from one phenophase to the next and in particular during shoot die-back.

2.2.2 Materials and Methods

2.2.2.1 Culture Conditions

P. angolensis seedlings were grown during December 2000 in the glasshouse of the Department of Forest Science at the University of Stellenbosch, South Africa. The mean monthly temperature in the glasshouse fluctuated greatly from a maximum mean monthly of 27°C to a minimum mean monthly temperature 6°C. Environmental control in the glasshouse was limited to cooling by two thermostatically controlled exhaust fans that were set at 35°C. The roofing for the glasshouse was made of fibre carbon glass that reduced solar radiation by 30%. Seasonal and diurnal ranges of solar radiation varied based on the seasonal and diurnal patterns obtaining in the Western Cape.

Seed obtained from Namibia at Hamoye (17° 56'S 20° 01'E) and was sown in 1.2*l* black plastic bags, with a depth of 30 cm, in sandy-loam soil with a pH of 6.9 (appendix Table 2.2.1). The potting mixture was made up of two parts sandy soil mixed with one part of fine compost. *P. angolensis* seeds were sown at a depth of 3 cm.

The experimental design was as follows
Replications = 5
Seed source = 1 (Namibia)
Number of seedlings per replication = 40
Sampling = Once in each of the 5 phenophases
Number of shoot apices sampled = 3 per replicate and per phenophase.

Phenological changes that occurred during the collection of shoot apices constituted the treatments. Five phenological changes that occurred between February and December 2001 constituted the five treatments and in each phenophase. Therefore shoot apices were collected in the months and during the phenological changes listed in Table 2.2.1. The November collection, which could have been made in October, was necessitated by the identification of a clear shoot apex.

Table 2.2.1. Phenological change and month for collection of shoot apices

MONTH	PHENOPHASE
February	Leaf expansion, shoot growth
March	Full leaf expansion & cessation of leaf growth; early stages of leaf yellowing
April	Leaf yellowing
June	No leaf and shoot growth, start of shoot die-back
November	Bud break, leaf emergence and opening, early phase of leaf expansion and shoot growth

Each bag received 70 ml water per day in summer and 35 ml every day in winter. Water was applied through over-head sprays twice each day in summer and once in winter. The study was conducted in a covered glasshouse with a uniform water regime but with varying temperature and light conditions that were influenced by the external environment.

A supplement as a source of nitrogen, ammonium sulphate, containing 21% N [210g/kg N (w/w)] was applied to each seedling at 10 ml of a 2 g/*l* solution on a weekly basis in the first season of growth. Thereafter the application was reduced to one application per month. Iron chelates (with 6% Fe) were mixed with water at 400 mg/*l* and applied at 1*l*/10m^2 of glasshouse space. The iron foliar spray was applied very lightly as chemical scotching occurred when the solution left a coating on the leaf after drying. Additionally seedlings were raised on a commercial fertilizer, Multifeed® which was applied as a foliar application. In all foliar applications a wetting agent was mixed with the nutrient solution at 1ml/5*l*. Non-nutritional glasshouse problems with the seedlings occurred with respect to aphids, mites and red spiders which were dealt with by the application of Metasystox R® at 2ml/*l* for aphids and mites, and cyhexatin 600 SC® at 1 ml/2*l* for red spiders.

2.2.2.2 Fixation, infiltration and embedding of shoot apices

Shoot apices were carefully cut with a sharp blade and fixed in FAA (Formalin-Aceto-Alcohol) which was made up of 5% formalin, 5% acetic acid and 90% of 50% ethyl alcohol. The vials contained sufficient FAA that facilitated the complete immersion of the shoot apex.

The dehydration and infiltration of plant material previously fixed in FAA was performed following the procedure in Table 2.2.2, modified from Johansen (1940).

Table 2.2.2. Dehydration and infiltration protocol for shoot apices previously fixed in FAA

STAGE	SOLUTION	MINIMUM TIME (in hours)	TEMPERATURE (°C)
1	Rinsing fixed material in distilled water 50% : ethyl alcohol 50%	3	Room temperature
2	Repeat of stage 1	3	Room temperature
3	Water 30% : Ethyl alcohol 70%	4	Room temperature
4	Water 25% : Ethyl alcohol 75%	4	Room temperature
5	Water 10% : Ethyl alcohol 90%	4	Room temperature
6	Water 0% : Ethyl alcohol 96%	4	Room temperature
7	100% tertiary butyl alcohol	6	Room temperature
8	100% tertiary butyl alcohol	6	Room temperature
9	100% tertiary butyl alcohol	6	Room temperature
10	Liquid Paraffin 50% : Tertiary butyl alcohol 50%	5	Room temperature
11	100% liquid paraffin	6	Room temperature
12	Liquid Paraffin 50% + Paraffin wax 50%*	12	65°C
13	Paraffin wax (100%)	96	60°C

** Paraffin wax was measured as 300ml weighing 165g (±3.1)*

The last two baths of the series in Table 2.2.2 consisted of thermostatically controlled stainless steel containers maintaining the wax and liquid paraffin and wax mixture at a constant melting temperature of 65°C and 60°C, respectively. Infiltrated shoot apices were individually placed in liquid paraffin wax in vials and cast in paraffin wax blocks.

2.2.2.3 Sectioning and staining

Embedded shoot apices were sectioned using a rotary microtome at 16µ. Sections were stretched in a water bath at 45°C to 50°C and mounted on microscope slides using Haupt's adhesive. Thereafter, tissues were further stretched and dried when they were placed, for up to 14 hours on a hot plate at about 50°C. Longitudinal tissue sections were pre-stained in 1% aqueous Safranin, in which the tissues were strongly stained, and then counter stained in Alcian Blue, as indicated in the series in Table 2.2.3.

Table 2.2.3. Staining protocol for *Pterocarpus angolensis* shoot apices

STAGES	SOLUTION	MAXIMUM TIME (minutes)
1	100% Xylene	5
2	100% Xylene	5
3	Water 10% : Ethyl alcohol 90%	2
4	Water 30% : Ethyl alcohol 70%	2
5	Water 50% : Ethyl alcohol 50%	2
6	1% Safranin	15
7	Water	Rinse
8	Alcian Blue	30
9	Water 50% : Ethyl alcohol 50%	1
10	Water 30% : Ethyl alcohol 70%	1
11	Water 10% : Ethyl alcohol 90%	1
12	100% Xylene	1
13	100% Xylene	Minimum 1

Stained specimens were mounted with DPX® and left to stand to allow the DPX microscopic mountant to solidify.

2.2.2.4 Microscopy and Analysis

Specimens were examined with a light microscope and measurements of the SAM (in microns) were made at 400X magnification. The following measurements and assessments were made:

- *Apical dome:* description of cell shape and arrangement, prominence of nuclei and availability of apical cell; the vertical distance (*a* in Fig. 2.2.1) from the apex of the apical dome to a point where a line drawn through the axil of the leaf primordia will cross at a right angle and the horizontal distance (*b* in Fig. 2.2.1) between the points of insertion of leaf primordia.
- *Tunica*: the number and thickness of cell layers *(e* in Fig. 2.2.1*)* in the tunica, the thickness was measured from the centre of the tunica and on either side of the apical dome closer to the leaf primordia axil and; number of cells in layers L1 and L2 in Figure 2.2.1. In all references to number of cells in tunica layers, the mean cell number for L1 and L2 is the parameter referred to.

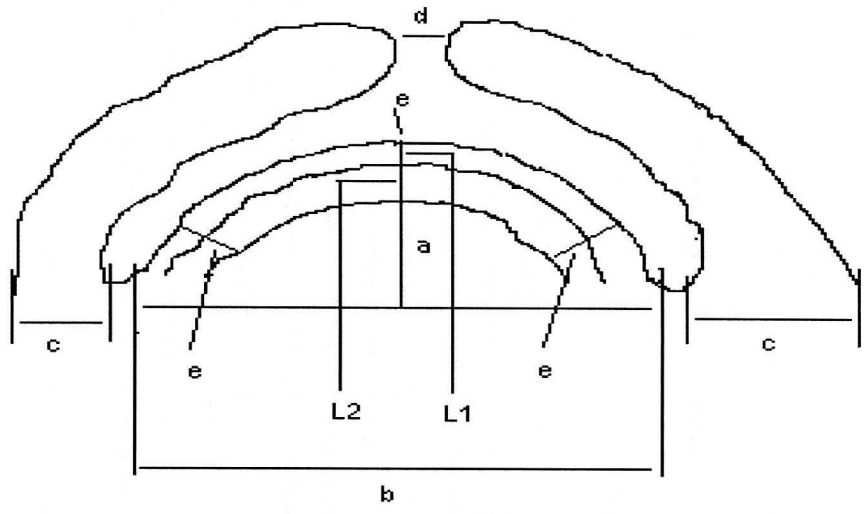

Figure 2.2.1. Reconstruction of a longitudinal section through the apical meristem showing a=apical dome vertical distance; b=diameter of apical dome; c=base of leaf primodium; d=gap between leaf primordia; e=points at which the thickness of L1 and L2 combined was measured; L1=outer tunica layer; L2=inner tunica layer.

- *Leaf primordia*: number of leaf primordia; whether the leaf primordia tips are above or below the apex of the apical meristem.
- *Protoderm:* description of cell size relative to tunica cells and number of layers that continue from the tunica cell layers.
- *Ground meristem:* general cell organisation and relative size compared to cells of the corpus; prominence of nuclei; differences from procambial cells, between the central zone and the two peripheral zones.

- *Procambium:* comparative cell structure and organisation to that of ground meristem; distance from apex of either apical dome or axil of youngest leaves and branching of procambial strand at the axil of the youngest leaf.
- *Older leaves*: The number of pairs of older leaves and whether these over-arch the apical meristem or not.
- *Ergastic compound*: Presence of ergastic substances in the ground meristem and procambium.
- *Apical dome volume:* Wagner's theoretical approach (Wagner, 2000) for determining volumetric values of mantle and core areas was applied. The apical dome was theoretically assumed to be a hemisphere, thus the following relationships are used

  $V_A = \frac{2}{3}\pi * r_A^3$

  Where V_A is the volume of the apex and r_A is the radius of the apex. The radius of the apex was taken to be

  $r_A = \frac{1}{2}(\frac{1}{2}b+a)$ refer to Figure 2.3.1. Therefore $V_A = \frac{2}{3}\pi * [\frac{1}{2}(\frac{1}{2}b+a)]^3$.

  The core volume was calculated as

  $V_C = \frac{2}{3}\pi * (r_A^3 - r_T^3)$

  Where V_C is the core volume or corpus volume and r_T is the mean of the thickness of the cell layers in the tunica ($r_T = \frac{1}{3}[\sum(e)]$) as in Figure 2.3.1.

  Therefore $V_C = \frac{2}{3}\pi * [(\frac{1}{2}(\frac{1}{2}b+a))^3 - (\frac{1}{3}\sum(e))^3]$ when related to Figure 2.2.1. The volume of the mantle is obtained by subtracting V_C from V_A, which departs from the Wagner (2000) direct computation of the mantle volume;
  an analysis of variance for SAM, corpus and tunica volumes as well as ratios of core and mantle to apical dome volume, and mantle to core volume was carried out to ascertain phenophasic variations.

Quantitative data was analysed using the general linear model in SAS Enterprise Guide Release 1.3 (SAS Institute, 2001).

Due to the fact that all replications did not yield specimens (Table 2.2.4), only an analysis of the regression was carried out. The linear relationships between seedling age and apical meristem properties (cell number, SAM diameter and volume, corpus and tunica size) were tested by the examination of the linear model $Y = a + \beta x + \beta x^2 + \varepsilon$ where Y is the response variable (apical dome traits) and x (seedling age or either of the apical dome traits) is the independent variable. SAM properties were also compared among themselves independent of seedling age.

Table 2.2.4. Number of specimens examined and used in the regression analysis of the apical dome traits and either seedling age or any apical dome trait

Phenophase	Replications (with specimens)	No. of shoot apices	TOTAL NUMBER OF SPECIMENS EXAMINED						
			No. of cells	SAM vertical distance	SAM diameter	SAM radius	Tunica Thickness	Corpus radius	Primordia height
Early leaf expansion	3	3	4	3	3	3	4	3	6
Late leaf expansion	3	4	7	6	6	6	10	6	11
Leaf yellowing	2	2	2	2	2	2	4	2	6
Shoot die-back	4	4	4	2	2	2	5	2	5
Leaf flush	2	2	2	2	2	2	3	2	1

The structure of the apical meristem of *P. angolensis* was detailed from qualitative observations. Other non-quantitative phenophasic differences were discussed together with results from quantitative analyses or separately depending on whether they refer to the same anatomical feature or not.

2.2.3 Results and Discussion

The results are presented graphically and in tabular form, the latter presentation method includes all shoot apical meristem parameters. The sizes and volumes for the shoot apical meristem, tunica and corpus were not linearly related to seedling age (appendix Table 2.2.2) whereas the number of cells in the tunica layers (appendix Table 2.2.3) was linearly related to all other variables with the exception of tunica thickness.

Characterisation of the structure of the shoot apical meristem of P. angolensis

The general structure of the longitudinal section of *P. angolensis* apical meristem is similar to the general structure of apical meristems of angiosperms. However, the longitudinal section of the shoot apex of *P. angolensis* shows a dome-shaped structure (Fig.2.2.2) consisting of three distinct cell zonations with cells dissimilar in shape, size and alignment. The mantle or tunica is made up of two layers of cells that are rectangular with the longest side orientated parallel to the surface of the apical meristem. The epidermal and sub-epidermal cell layers, forming the tunica, are characterised by conspicuous nuclei, apparent lack of vacuoles and a deep blue stain. The two tunica cell layers enclose a region of cells, the corpus, that are not orientated in any particular order and not rectangular in shape. Both the tunica and corpus are characterised by a dense cytoplasm and prominent nuclei are also characteristic.

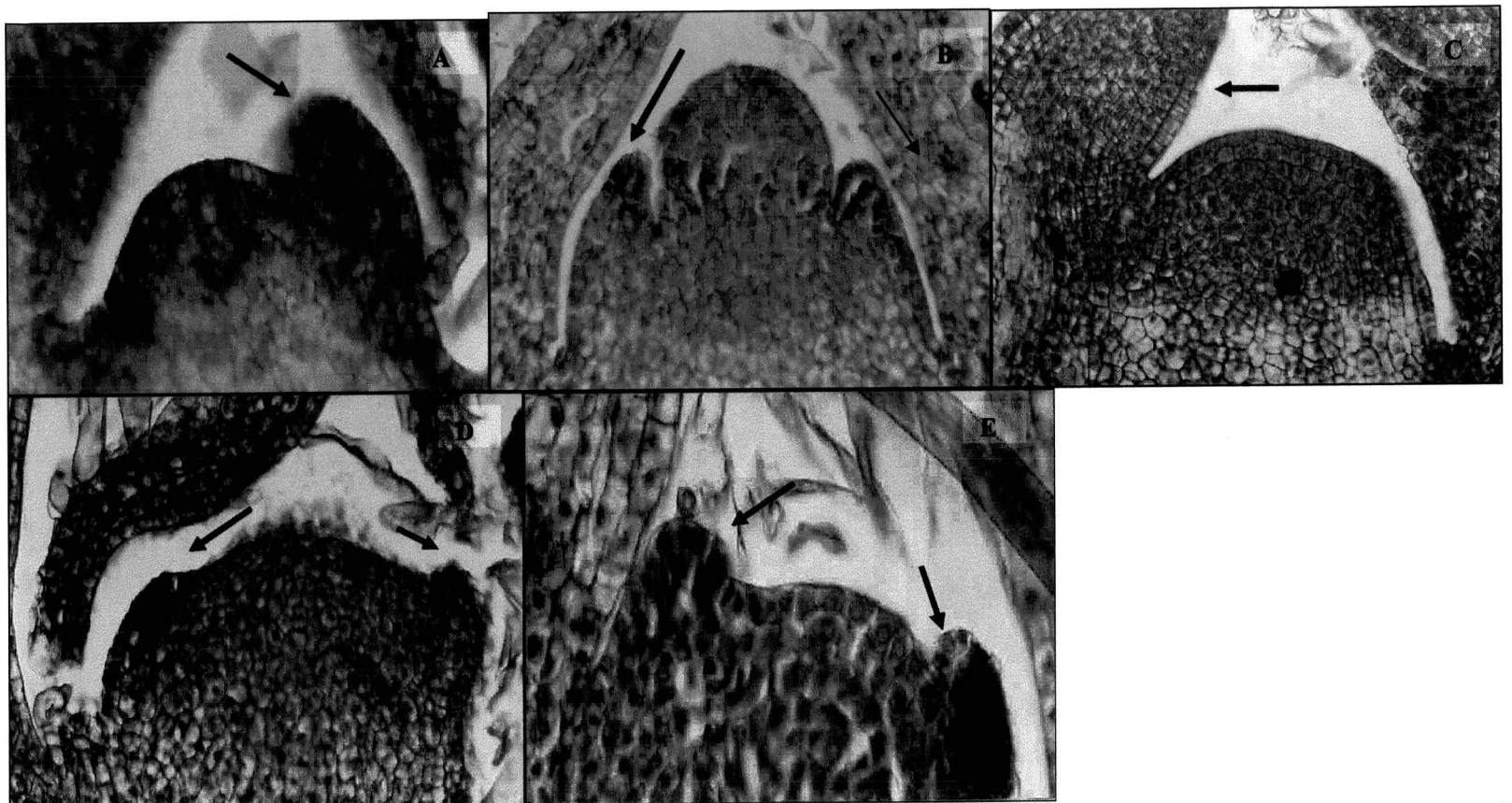

Figure 2.2.2. Longitudinal section of the shoot apex of P. angolensis. The apical meristem is surrounded by developing leaf primordia (arrows) in different stages of development. The L1 and L2 cell layers in the mantle (tunica) are clearly visible in (C) during late phase of leaf expansion and early stages of leaf yellowing. Each SAM represents a phenophase – A = leaf flush; B = leaf expansion; C = late leaf expansion and early leaf yellowing; D = leaf yellowing and E = shoot die-back or dormancy. (scale 10 mm = 21 µm)

One or two leaf primordia are found at the sides of the apical dome at all stages of growth. Leaf primordia may or may not be arched over the apical dome.

A mass of trichomes occur in the abaxial epidermis of the young leaves. Cells of the ground meristem are hexagonal with large vacuoles. Procambium strands run parallel on either side of the terminal shoot just below the apical meristem. In some cases branching of the procabium strands to the leaf primordia is present. Closely packed elongated procambium cells form two separate strands along the central region of ground meristem. Cells of the procambium strands have prominent nuclei and are stained a slightly darker blue than the cells in the ground meristem.

Dimensions of the shoot apical meristem

The number of cells in the tunica was linearly related to the age of the plant (Figure 2.2.3a). The number of cells in the tunica was also observed to be significantly linearly related to SAM vertical distance, SAM diameter, SAM radius and corpus radius with the exception of tunica thickness (appendix Table 2.2.1). SAM, corpus and tunica volumes as well as primordium height were significantly related to the number of cells in the tunica (appendix Table 2.2.2). A decline in cell number is apparent during the period of shoot die-back with apical meristems at leaf flush possessing the highest number of cells in the tunica layers (Table 2.2.5).

Table 2.2.5. Table of means for shoot apical meristem, tunica and corpus' radii and volume (sizes in μm)

SAM Variable	SEASON				
	Leaf flush	Leaf maturation	Leaf maturation/ yellowing	Leaf yellowing	Shoot die-back
Cell number in tunica	23(±0.50)	22(±1.67)	22(±1.83)	22(±4.78)	13(±1.24)
Tunica thickness	293(±9)	281(±9)	313(±11)	326(±37)	322(±22)
Tunica volume	9.93E+8 (±3.94E+8)	10.9E+8 (±1.69E+8)	9.92E+8 (±1.8E+8)	14.1E+8 (±2.92E+8)	20.3E+8 (±14.8E+8)
Corpus radius	558(±145)	632(±77)	542(±63)	664(±113)	555(±241)
Corpus volume	5.21E+8 (±3.69E+8)	6.34E+8 (±1.57E+8)	4.96E+8 (±1.39E+8)	8.71E+8 (±3.15E+8)	13.7E+8 (±11.8E+8)
SAM radius	850(±142)	910(±73)	829(±59)	979(±104)	829(±267)
SAM volume	15.1E+8 (±7.63E+8)	17.2E+8 (±3.24E+8)	14.1E+8 (±2.83E+8)	22.8E+8 (±5.61E+8)	30E+8 (±23.8E+8)

Seasonal changes in cell number in the tunica layer of *P. angolensis*, with particular reference to shoot die-back, may be indicative of dimunition in apical growth, particularly declined frequency of plastochronic changes. Frequencies in leaf initiation decline from the season of leaf yellowing and completely cease with the onset of shoot die-back. The secession of leaf initiation is observed from a shoot tip which becomes fluffy and brown. As expected, the decline in leaf primordia formation may follow gradual and ordered senescence processes, probably preceded or accompanied by a decline in the mitotic activity of the apical meristem. Dimunition in apical size generally reflects the decline in the whole apical volume that precedes shoot die-back and is facilitated by a decline in cell number in the tunica (Fig. 2.2.3). In *Picea mariana* (Mill) B.S.P. and *Picea glauca* Moench Voss, and *Pseudotsuga menziensii* (Mirb.) Franco var. *menziesii* seedlings, MacDonald (2000) found a decline in mitotic activity corresponding to leaf initiation. Dividing cells were only found localised in the apex proper, in a region distal to the youngest primordia, in dormant buds of the garden pea (Stafstrom, 2000).

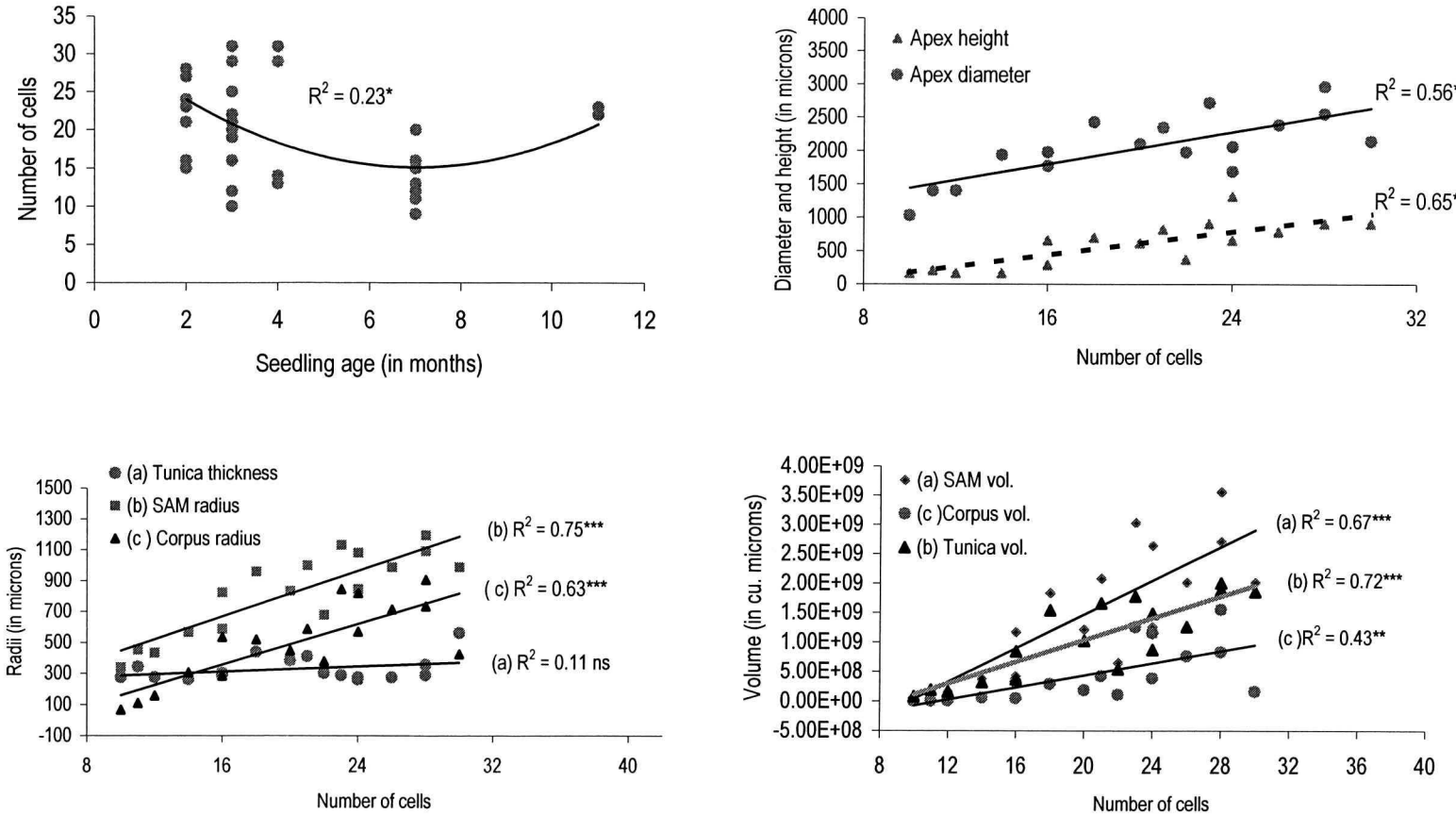

*Figure 2.2.3. Relationships between seedling age and the number of cells in the tunica layer as well as those between the number of cells in the tunica with the radii and volumes of the apical dome, corpus and tunica. R^2 followed by *, **, *** and ns are significant at 5%,1%, 0.1% and not significant respectively.*

SAM, tunica and corpus volume increase during shoot die-back probably due to declined frequency of leaf primordia initiation (Table 2.2.4). Generally, SAM diameter declines minimally during the period of shoot die-back when the transitory phenophase between leaf maturation and leaf yellowing is considered as a part of the leaf yellowing phenophase. The frontiers of such transitory phenophases are rarely well defined in nature.

Seasonal changes in leaf primordia height and breadth

Leaf primordia height and axil thickness declines towards the dormancy period (Fig. 2.2.4) which may be due to declined apical meristem growth. Leaf primordia height and breadth may not be sufficiently used as indicators of seasonal changes in the apical meristem due to the fact that primordium height and breadth vary based on the time of initiation. But Reinhardt *et al.* (2000) found that applying IAA to the site of incipient primordium formation resulted in increased primordium formation at the site. It was found that the base of the primordium was enlarged to an extent that it was thicker than the next older primordium. This implies that more cells were involved in primordium initiation than is normally the case due to the influence of IAA. It may be inferred that the decline in the morphometric properties of leaf primordia during the period of shoot die-back is influenced by the plants declined relative growth rate. Also, other internal factors such as the ratio of cytokinins-auxins and other chemical substances whose activity is strongly influenced by the environment, such low temperatures that apply to shoot die-back in *P. angolensis,* may be involved in determining leaf primordium size.

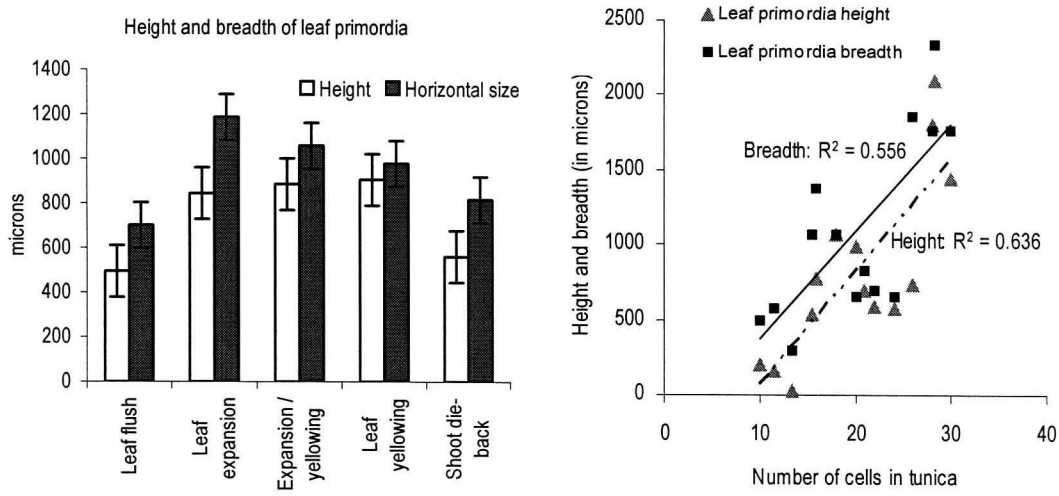

Figure 2.2.4. Leaf primordia height and breadth at the point of insertion, at the axil, and the linear relationship between leaf primordia size and the mean number of cells in the tunica.

The significant relationship between morphometric properties of leaf primordia and the cell number (appendix Table 2.2.3) may be related to the findings of Stafstrom (2000) who observed dividing cells being localised in the apex proper, a region distal to the youngest leaf primordia of garden pea dormant buds. The lack of cell division in the region proximal to the leaf primordia may actually result in a trend similar to the one in Figure 2.2.4 in which the growth and development of leaf primordia is restricted due to the reduced number of meristematic cells that have a symplasmic connection with the rest of the apical meristem. Probably the absence of symplasmic pathways between the leaf primordia and the rest of the plant affects also the balance of cytokinin-auxin resulting in a lower ratio that negatively impacts on its growth and development. A chemical factor or the relative proportions of chemical substances in combination with an environmental cue, such as low temperature, may determine the developmental pathway of leaf primordia resulting into bud-scales instead of foliage leaves. Signals originating from outside the meristem impose changes on the fate of the products of a meristem that can lead to bud-scales and not foliage leaves (Sussex, 1989). Temperature was identified as one of the signals imposing changes on the developmental fate of meristem products (Sussex, 1989; Kerstetter and Hake, 1997).

Presence of ergastic compounds in P. angolensis

Spherical solid particles, or ergastic substances, were found to occur throughout the annual cycle of *P. angolensis* and were principally concentrated in cells of the ground meristem but occasionally in those of vascular bundles. Spherical solid particles were found in the centre of the cell and rarely closer to the cell walls (Fig. 2.2.5) and the concentration of solid particles was not tissue specific. Ergastic substances can or cannot be found in the plant at different times of its life cycle and their concentration and size may be influenced by elevated CO_2 and nitrogen concentration, respectively (Esau, 1965; Utriainen and Holopainen, 1998; Utriainen and Holopainen, 2001). Solid particles have been reported to be most commonly made up of starch grains that develop within plastids (Esau, 1965; Fahn, 1969). In young dicotyledonous stems, the innermost cortical layer usually contains many starch grains.

Figure 2.2.5. Ergastic compounds in cells of P. angolensis seedling longitudinal sections of shoot apices. In (A) starch substances, at leaf yellowing, are clearly visible and mainly concentrated in the center of the cell and most likely in the cell vacuole. The thick cell wall includes a thin layer of cytoplasm. (B) Prismatic crystals of various shapes (refer to arrows) occurring singly in each cell during leaf expansion. Crystals were observed to be concentrated on margins of vascular bundles and rarely in the cells surrounding vascular bundles. (C) Crystal compounds at leaf expansion, which rarely occurred in this species, are clustered in near spherical masses forming druses. (D) Prismatic crystals are concentrated in cells occurring on the margins of vascular bundles during shoot die-back, in phloem and xylem cells and none were observed in the procambium, whilst starch grains are mainly located in the cells of the pith or ground meristem. (scale: 10 mm = 15 µm

The smallest of the particles found in cells nearer to SAM may be related to the age of the cell. They are slowly pushed down with differentiation and growth where they keep on growing in size and become aggregated. Storage of starch in living cells of the deciduous *P. angolensis* during the growing season is for use as energy for regrowth in the next growing season. This may be the reason why starch grains were observed throughout the phenophases of this species.

Prismatic crystals occurring on the edges of vascular bundles and within cells of the primary xylem and phloem were observed to occur only in the tissues located on the fringes of vascular bundles and not in the procambium. The crystals were transluscent and occurred singly in each longitudinal cell of the primary vascular tissue. The major difference in location between spherical particles and crystals was in their sizes, shapes and location within the plant body. All tissues from all phenophases had prismatic crystals whereas the season of leaf flush had a small proportion of tissues containing prismatic crystals (43%). This may be due to the fact that sampled shoot apices, during leaf flush, were current season's growth and therefore contained very young tissues which had as yet not formed crystals.

2.2.4 Conclusion

Assessments of seasonal changes in anatomical characteristics of the shoot apical meristem have indicated the link between the decline in cell number in the tunica with the period of shoot die-back. Other characteristics of the shoot apical meristem have not been been linked to the phenology of *P. angolensis*. Dehydration and infiltration protocols used for the preparation of *P. angolensis* shoot apices for microtomy and staining procedures were applied successfully for the first time in this species, if not the genera. The development of these protocols partly contributed to the failure to obtain a sufficiently replicated sample for the analysis of variance.

The lack of a significant relationship between the volume of the shoot apical meristem and phenophases points to uniformity in the seasonal size of the shoot apical meristem. Even though cell size was not measured due partly to the size of the tissues, 16μ, the decline in cell number has a direct effect on the volume of the corpus, tunica and shoot apical meristem. Interestingly enough, leaf primordia height and breadth declined with cell number.

The seasonal decline in cell number, particularly during shoot die-back, is linked to declined mitotic activity in the proximal zone to the leaf primordia which results in the reduction of cell number of the whole apex. Since the developing leaf primordium orients cell division towards itself, the absence of, or a decline in mitotic activity in the proximal zone to the leaf primordium, may directly affect its size or basic result in non-initiation of leaf primordia.

The presence of ergastic compounds, particularly the prismatic crystals, may have a systematic nature as well as a seasonal one. Consistently, similar structures of crystals occurred over the tissues, particularly in areas proximal to vascular bundles. A small proportion of tissues contained crystals during the period of bud break and leaf flush. Since prismatic crystals have a calcium oxalate origin and high concentrations of calcium are linked to marked decreases in ethylene production and the activation of calmodulin, depending on the regulatory mechanisms in plants, there might exist a seasonal differential in the concentration or sizes of the crystals and their relative location or abundance from young tissues of *P. angolensis*. Further experimentation is required to link the seasonal decline in cell number with shoot apical volume parameters, sizes of leaf primordia and seasonality in occurrence of prismatic crystals from the shoot apical meristem.

2.3 PHENOPHASIC MINERAL NUTRIENT CONCENTRATION IN *Pterocarpus angolensis* SEEDLINGS

2.3.1 Introduction

Phenophasic (phenological or seasonal) changes, occurring in *Pterocarpus angolensis* are either influenced by an environmental cue or genetic factors and are accompanied by changes in relative tissue mineral nutrient concentrations. The major phenological change occurring in this species is shoot die-back, a form of organ senescence. Mobile ions have been found to move out of senescing tissues leading to an extensive export of minerals (Sabater *et al.*, 1990b). The major proportion of mineral nutrients accumulate in the leaves, which may vary considerably with age and position on the tree (Kramer and Kozlowski, 1979; Drechsel and Zech, 1993) and the availability of nutrients (Van de Driessche, 1984). Therefore the remobilisation of components during leaf senescence is essential for nutrient economy (Sabater *et al.*, 1990b). In this regard, it has been observed in current foliage of *Pinus radiata* that low concentrations of nitrogen and phosphorus in older needles occurred only on infertile sites (Van de Driessche, 1984). Remobilisation of ions depend on their mobility, which varies with the species, the stage of growth and the amount of the element in the plant, and is partly determined by the solubility of the chemical form of the element in the tissue (Salisbury and Ross, 1985; Drechsel and Zech, 1993; Mallik and Timmer, 1998). The remobilisation of ions makes necessary the activation of several hydrolytic enzymes and other metabolic activities that are mainly associated with amino acid metabolism which form low size exportable nutrient molecules (Kramer and Kozlowski, 1979). The phytohormones that appear to be associated with nutrient remobilisation have been reported to be cytokinins (Taiz and Zeiger, 1998).

The continuous nutrient traffic ensures the recovery of a significant portion of nutrients from old structures to young developing organs. Young and developing plant parts have a pronounced ability to withdraw mobile nutrients from older organs (Bidwell, 1979; Drechsel and Zech, 1993).

Seasonal changes in nutrient cycling are highest during the growth and less in the dormant season (Kramer and Kozlowski, 1979). This reflects a higher physiological requirement for the retranslocated nutrient in the rapidly developing destination organ. In *Quercus coccinea,* the retranslocation of nitrogen, phosphorus and potassium was observed by Van de Driessche (1984) to occur from mid-September till leaf abscission, whereas in *Fagus sylvatica* about 45% of nitrogen and potassium was withdrawn from the foliage between late September and leaf fall. Essential mineral elements are broadly classified as mobile (magnesium, nitrogen, phosphorus, potassium), immobile (boron, calcium and iron) and intermediate (copper, manganese and zinc). Even though nutrient remobilisation depends on the relative mobility of ions, their availability to and concentration in the plant are influenced by the soil pH, oxygen content and the ion exchange capacity of the soil.

In situations where a particular element becomes deficient, such as when an element is retranslocated to young and developing plant parts, nutrient deficiency symptoms may occur. The nutrient deficiency symptoms are the expression of physiological disorders arising from the inadequate availability of an essential element (Drechsel and Zech, 1993; Taiz and Zeiger, 1998) and these become manifest first in young or older organs. The commonest nutrient deficient symptom in seedlings, which is also common in *P. angolensis*, is the chlorosis of the foliage. Chlorosis is caused by nutrient deficiencies that facilitate the unavailability of an element or elements required in the synthesis of chlorophyll and enzymes as well as the prevention of chlorophyll degradation. In *P. angolensis* chlorosis has been reported to be partly caused by iron deficiency which also caused poor root development (Munyanziza *et al.*, 1998). Even though chlorosis is mainly associated with the lack of nitrogen, it is also induced by deficiencies of iron, magnesium, manganese, potassium and other elements (Bidwell, 1979; Kramer and Kozlowski, 1979; Salisbury and Ross, 1985; Taiz and Zeiger, 1998). Due to the fact that the visible effects of nutrient deficiency cannot be associated with only the role of a single nutrient, other nutrients and external factors may also be involved. Numerous unfavourable environmental factors including an excess or deficiency of water, unfavourable temperatures and an excess of chemical substances as well as genetic factors can induce chlorosis and other physiological and morphological changes in plants (Kramer and Kozlowski, 1979).

Other factors may include leaf aging before leaf shedding, diseases or a combination of nutrient deficiencies followed by virus attack (Drechsel and Zech, 1993).

The experiment was carried out to ascertain the mineral nutrient concentration in each phenophase in *P. angolensis* seedlings over two growing and two dormant seasons. It was hypothesised that the phenophasic changes in mineral nutrient concentrations were similar in all phenophases for leaves, stems and roots.

2.3.2 Materials and Methods

Seed from Hamoye in Namibia (17° 56'S 20° 01'E) was used to raise seedlings in the Department of Forest Science nursery of the University of Stellenbosch, South Africa. Seeds were sown and germinated in December 1999. The conditions in the nursery were as described in 2.1.2. Seed was sown in 1.2 *l* black plastic bags, with a depth of 30 cm, in sandy-loam soil with a pH of 6.9 (appendix Table 2.2.1). The potting mixture was made up of 2 parts sandy soil mixed with one part of fine compost that was inoculated with soil containing mycorrhiza from a *P. angolensis* site near Nelspruit. *P. angolensis* seeds were sown at a depth of 2-3 cm. Each bag received 70 ml of water per day in summer and 35 ml every other day in winter. Water was applied through over-head sprays thrice each day in summer and once in winter. Fertilisers were not applied to the seedlings nor to the soil.

Replications = 3
Seedlings per replication = 100
Number of sampling times = 4 (corresponding to 4 phenophases)
Number of seedlings sampled per phenophase = 10 per replication

Each of the three samples, with 10 plant parts each, were taken every time a significant phenological change was observed over a period of 17 months that spanned two growing and two dormant seasons. Sampled seedlings were subdivided into leaves, stems and roots. Each tissue type separately in plastic bags and analysed. The samples were taken between 8:00 and 10:00 on each day of sampling. Sampling of plant tissues was carried out five times over seventeen months.

The low number of samples was necessitated by the insufficient number of seedlings which had leaves during the time of shoot die-back. Phenophases at which sampling took place were time of leaf flush, full leaf expansion (vivid green colour), yellowing of foliage (generally characterised by a dull chlorotic yellow green colour typical of nutrient deficiency) and period of shoot die-back (characterised by chlorosis and necrosis in leaves which are later followed by a shrunken shoot apex and gradual shoot die-back from the shoot apex towards the root collar). The sampling at shoot die-back, between June and July, only considered seedlings that had leaves on them and not the ones without leaves. Therefore this meant that subsequent samples, after the first growing season, used leaves and stem from that season's growth.

Ten essential mineral elements were determined at INFRUITEC in Stellenbosch - boron (B), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), nitrogen (N), phosphorus (P), potassium (K), and zinc (Zn). Leaching losses for cations that are very susceptible to leaching, such as Mg and K, were not determined.

The general model for the analysis was $Y_{ijk}=\mu+R_i+ S_j + T_k+\varepsilon_{ijk}$, where Y_{ijk} is the response obtained at the i^{th} replicate, j^{th} phenophase or season and in the k^{th} tissue type, μ is the mean of the population, R_i is an effect due to the i^{th} replication ($i = 3$), S_j is an effect due to the j^{th} season when sampling is carried out ($j = 4$), T_k indicates an effect due to the k^{th} tissue type ($k = 3$) whereas ε_{ijk} is a random error term associated with main effects. The ε_{ijk} are assumed to be normally distributed, with mean 0 and unknown variance, and independent. When seedling age was considered instead of the season, S_j was taken as an effect due to the j^{th} age with j equalling six seedling ages. The analysis of variance within tissues for all seasons was based on the general linear model $Y_{ij}=\mu+R_i+ S_j + \varepsilon_{ij}$, with the terms explained similar to the earlier model with the omission of tissue types.

The association between mineral nutrients, as response variables of interest, and the seedling age at which assessments were carried out was summarised by fitting and assessing the quality of fit of the simple model $Y_i=\beta_0+\beta_1 x+\varepsilon_i$ (for *Ca, Mn, Cu, Zn* and *B*), where β_0 is the intercept, β_1 is a measure of the rate of increase in a mineral nutrient with seedling age and ε_i are error terms that represent the vertical deviations of the i^{th} observed value from the fitted line.

The standard error of the estimate of β_1 was used to determine the significance of the *t* statistic in testing the absence of a dependence of mineral nutrient concentration on seedling age. The lack of fit of the assumed linear model was tested using the residual mean square, an estimate of variance, in the analysis of variance of the regression by breaking down the residual mean square. Repeat mineral nutrient concentrations at each of the seedling ages, three times for each age, were used to calculate one component of the residual mean square (the true estimate of σ^2 that represents "pure error") and the remaining component of the residual mean square was assumed to be due to the failure of the model to fit the data – it represents the lack of fit.

The univariate analysis among the phenophases, $\alpha = 0.05$, for foliage, stem and root nutrient concentrations, and sampling times for the root, was performed using the general linear model procedure in SAS (SAS Institute, Inc., 1996) and the graphical presentations prepared in MS Excel 2000 (Microsoft Corp., 1999). Tests of homogeneity between means were performed with the Student-Newman-Keuls test.

Graphical presentations, for relative seasonal changes in nutrient concentrations, were made assuming that the pool of nutrients at full leaf expansion is the highest for the plant, and that the changes observed in the nutrient pool are changes that are relative to the original nutrient pool. Therefore, to determine the phenophase-related nutrient changes, all calculations of differences between mean nutrient concentration for each season were related to the nutrient pool at full leaf expansion. All micro-nutrients had the highest mean nutrient concentration at full leaf expansion with the exception of boron which had the highest concentration at leaf flush. The relative change (RC) in nutrient concentration was calculated as

$$RC = \frac{NC_{CS} - NC_{le}}{NC_{le}}$$

NC_{cs} – nutrient concentration in current season's tissue;
NC_{le} – nutrient concentration at full leaf expansion.

2.3.3 Results and Discussion

Results are presented graphically and in tabular form. Detailed discussions and illustrations are presented for mineral nutrients that exhibited significant differences amongst the seasons, tissues and seedling ages, whilst mineral nutrients that showed no significant differences amongst seasons, tissues and seedling ages are not discussed at length. Appendices contain tables of the complete analyses of variance and comparison of means. Only pairs of mineral nutrients that had higher and significant correlations in specific phenophases (appendix Table 2.3.5) were further examined to determine the form and strength of relationship over the phenophases in which the correlations were higher and significant.

2.3.3.1 Seasonal and plant tissue changes in macro-element concentrations of *P. angolensis* seedlings

Macro-elements that exhibited significant seasonal effects were *N, P, K* and *Mg* (appendix Table 2.3.2). Phenophases differed significantly in terms of foliar *N, Ca* and *Mg* concentrations. Similarly, significant variations were obtained amongst the phenophases with respect to the concentrations of *Mg, P* and *K* in the root. The concentration of foliar *N* was significantly lower during shoot die-back than in other seasons whereas the concentration of *Ca* rose significantly to a maximum during leaf expansion and declined at leaf flush (Fig. 2.3.1). The mean root *P* concentration at leaf flush differed significantly from the rest of the phenophases but declined towards shoot die-back (Figure 2.3.1). A significant decline in root *K* concentration was observed between leaf expansion and shoot die-back whereas this occurred a season early, between leaf flush and die-back, for *Mg*.

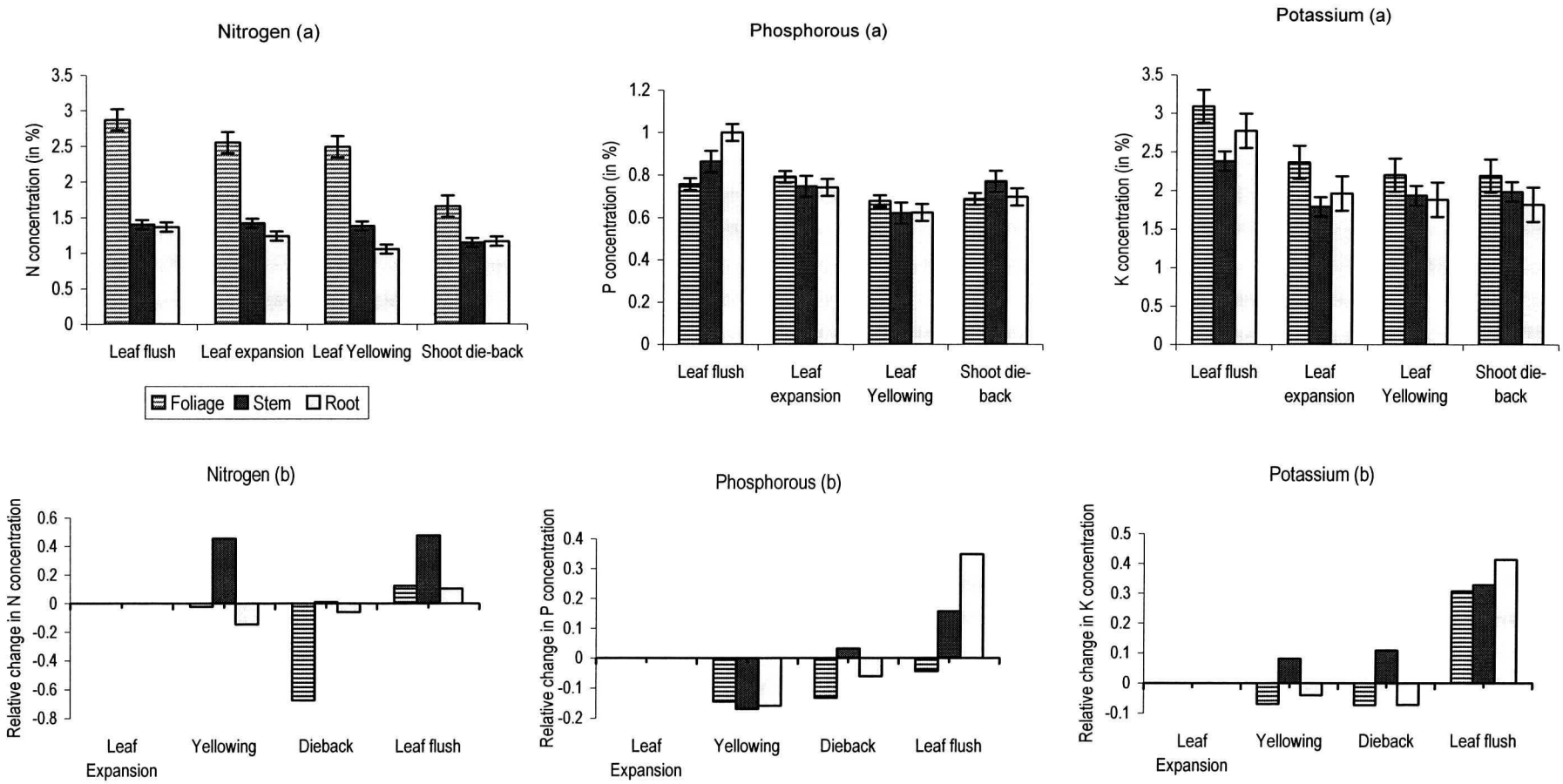

Figure 2.3.1. Phenophasic mean concentration of N, K and P in foliage, stem and root. Mineral elements followed by suffix (a) indicates the mean phenophasic mineral nutrient concentration for N, K and P in the foliage, stem and root whereas those followed by (b) are relative changes in mineral nutrient concentration in the three tissues of P. angolensis seedlings over four growth seasons. Leaf expansion refers to the season when leaves reach full maturation.

The decline in foliar *N* concentration during shoot die-back contrasted with the low relative increase in root *N* concentration (Fig. 2.3.1) during the same phenophase. Concentrations of foliar *N* were higher during the initial phases of growth than during the period of shoot die-back. Lower *N* concentration occurred during the period of shoot die-back in which leaf expansion is least expected, which was also observed by Lambers *et al.* (1998). The significant decline in foliar N may correspond to the fact that the photosynthetic capacity of the foliage of the species may decline during shoot die-back, as inferred from the yellowing and stunted pinnae. One of the key factors determining the existence of a relationship between maximal photosynthetic rate and leaf N and P (Trebst, 1994; Misra *et al.*, 1998; Cherbuy *et al.*, 2001) is increasing N availability through fertilisation. Secondly, since maximum assimilation rates are expected to correlate strongly with photosynthetic capacity (Rood *et al.*, 2000), leaf age effects in *P. angolensis* may have had confounding stomatal limitations due to the fact that the average lifespan of leaves in this species is from leaf flush to the beginning of shoot die-back. Naturally there is a complete shedding of leaves in seedlings, but between 10 and 30% of the foliage remains on the plant in the glasshouse due to high moisture levels during shoot die-back. The surviving leaves are usually rarely healthy but chlorotic which may have an effect on the allocation patterns of leaf N. The release of axillary buds ceases in *P. angolensis* seedlings during the period of leaf chlorosis, shedding or necrosis and shoot die-back during which low concentrations of foliar *N* are obtained. This observation is supported by Kramer and Kozlowski (1979) and Schulze *et al.* (1991) who indicated that low concentrations of *N* lead to a reduction in final leaf size, release of axillary buds as well as the accumulation of starch.

The probable reason for the increase in foliar *Ca* concentration during shoot die-back may be related to its role as a co-factor in some enzymes involved in the hydrolysis of ATP and phospholipids. The phenophasic concentrations of foliar *Ca* are almost the inverse of the root *Ca* concentration except that the highest relative decline in root *Ca* levels is during the period of leaf chlorosis and shoot die-back (Figure 2.3.2), then rising through leaf flush. An increase in hydrolytic enzyme activity may be required during this phase in order to break down macro-molecules such as proteins into smaller and translocatable molecules, such as amino acids, for storage in the root that has longer residence times for nutrients.

The reason for foliar *Ca* increase may be inferred from its role in plant senescence in which it plays a major role in membrane structure and function. Calcium plays an important role in the regulation of ion transport, maintenance of RNA and protein levels (Poovaiah, 1988). High levels of free Ca^{2+} in the cytoplasm is injurious to the cell because it reacts with inorganic phosphate to form an insoluble precipitate. Therefore in situations where cytosolic Ca^{2+} concentrations are allowed to reach the millimolar levels present in the extracellular space, phosphate-based energy metabolism will be seriously inhibited (Poovaiah, 1988). *Ca* plays dual roles in plant senescence, i.e. marked senescence delaying effects and hastens senescence and results in death.

A marked decrease in ethylene production, one of the hormones that is involved in leaf blade senescence (Mattoo and Aharoni, 1988), has been associated with the application of calcium either as $CaCl_2$ or $Ca(NO_3)_2$, which has been linked to some degree of inhibition of oxidative metabolism (Frost, 1986). Depending on regulatory mechanisms in plants, internalised *Ca* has been associated with promoting senescence by activating calmodulin (i.e. calmodulin mediates phospholytic and subsequent lipoxygenase activity on cell membranes) and when *Ca* is externally situated it maintains senescence deferral (Frost, 1986; Taiz and Zeiger, 1998). Therefore, the foliar *Ca* increase observed in *P. angolensis* during shoot die-back may either have been to hasten or delay senescence. The problem underlying the assumption of the probable role of *Ca* in this case, is the length of the senescence event in individual plants, it does not occur within a specific week or month in all plants.

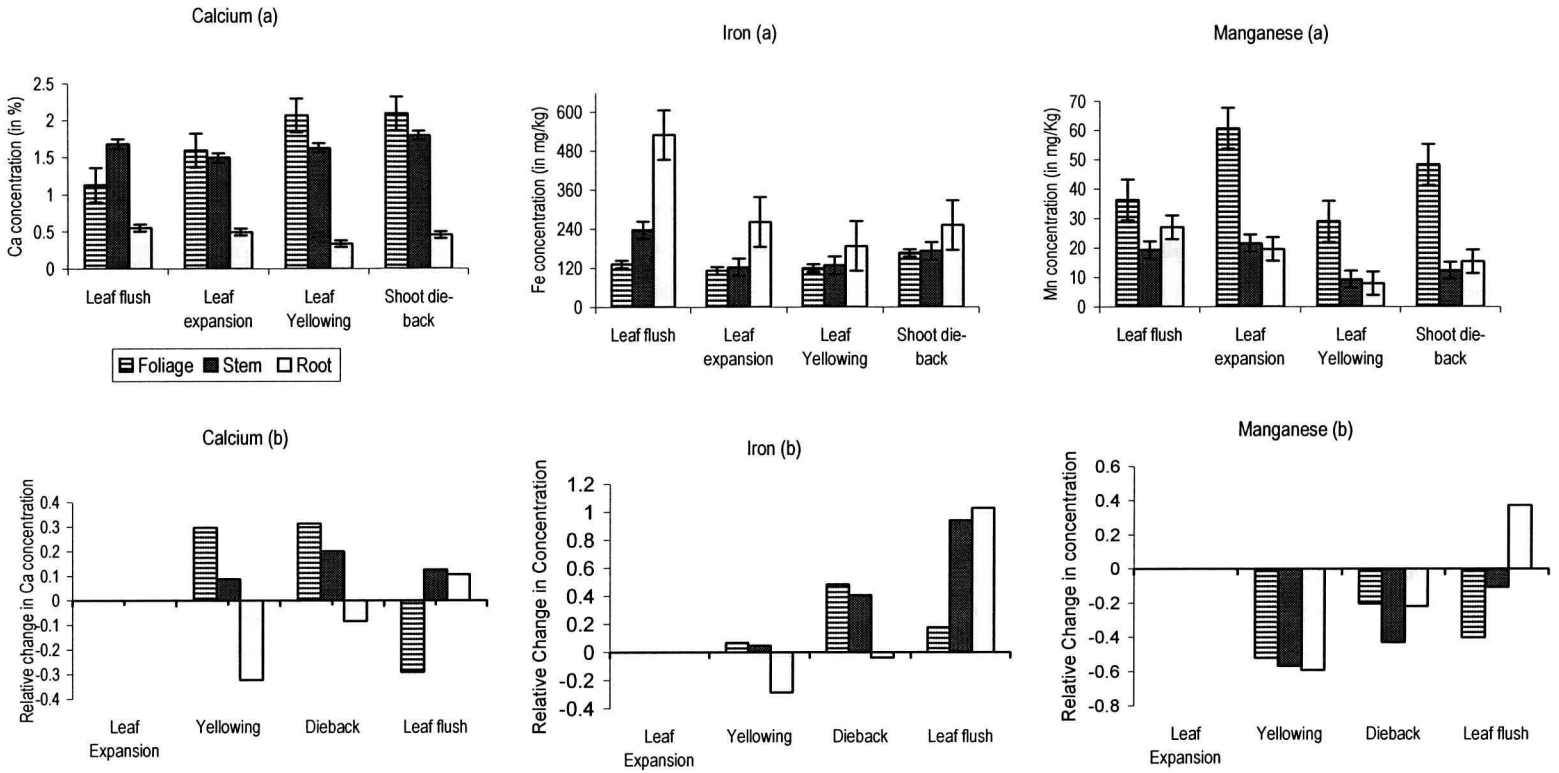

Figure 2.3.2. Phenophasic mean concentration of Ca, Fe and Mn in foliage, stem and roots. Graphs labelled (a) illustrate the mean phenophasic mineral nutrient concentration in the foliage, stem and root whereas those labelled (b) show relative changes in mineral nutrient concentration of P. angolensis seedlings in the three tissues over four growth seasons. Vertical bars in the (a) graph series signify the standard error for each tissue type. Leaf expansion refers to the season when leaves reach full maturation.

Since the leaves are invariably supposed to have been shed by then, it may be inferred that the increase in foliar *Ca* had a senescence deferral effect. This can also be deduced from the fact that *Ca* generally increased in all plant tissues.

The phenophasic changes in concentration of *P* in the root was significant even though no significant seasonal changes in concentration were observed in the foliage and stem (appendix Table 2.3.2). Relative changes in concentration of root *P* increased dramatically after shoot die-back to the leaf flush season (Fig. 2.3.1). The low *P* concentration during shoot die-back in the foliage and roots may be due to its high mobility, which may result in lateral bud dormancy (Bidwell, 1979) as well as chlorosis (Taiz and Zeiger, 1998). In *Eucalyptus* species, Van den Driessche (1984) found that 70-80% of *P* may be retranslocated from the leaves before leaf abscission. Even though the decline in foliar *P* was only 13% from leaf expansion to shoot die-back, Bielesk (1973) indicated that net losses of *P* can lead to a 50% decline in foliar concentrations of *P* in senescing leaves.

Potassium concentration significantly varied across phenophases in the root but not in the foliage and stem (appendix Table 2.3.2). In Figure 2.3.1, the relative decline in the level of *K* in the foliage and root is highest during shoot die-back but increases sharply with the onset of growth. The foliage contained the highest concentration of *K* during leaf flush, which was not significant over the phenology of the plant (appendix Table 2.3.3). The maintenance of high levels of *K* over time, regardless of phenophases, may be due to the important role of *K* as the principal cation that maintains cell electroneutrality and establishes cell turgor. The fact that it indirectly hinders carbohydrate translocation and nitrogen metabolism (Kramer and Kozlowski, 1979), shows the need for the continuation of metabolic activities such as the synthesis of energy compounds required in the control of leaf senescence. Since seedlings undergo shoot die-back during this season in which *K* declines, the need to maintain solute concentrations in the root are high and *K* is indispensable due to its role in maintaining the water balance of the plant. The foregoing is physiologically necessary due to the fact that the absence of the shoot or physiological inactivity in the stem and leaves are likely to lead to the accumulation of mineral elements in the root.

The seasonal differences in root *Mg* concentrations during leaf expansion and yellowing may be indicative of the apparent high demand for *Mg* and the problems of translocation, respectively. Since the deficiency of *Mg* causes chlorosis and disintegration of ribosomes, the higher *Mg* concentration in all tissues during leaf flush and less towards shoot die-back may be explained by its activation of enzyme systems involved in phosphate transfer.

With the exception of iron and phosphorus which are higher in the root than the foliage when all seasons are considered together (appendix Table 2.3.4), higher concentrations of *N, P* and *Ca* in the foliage of *P. angolensis* seedlings partly affirm the observation of Drechsel and Zech (1993) that the highest concentration of *N, P, K* and *Mg* in tropical conifers and broadleaved species is in the foliage. The higher concentration of *N* in the foliage, particularly during leaf flush and expansion, is necessary since it promotes shoot growth relative to root growth whereas the opposite applies in *P* concentration which is higher in the root than in the rest of the plant. This may be the reason why *N* declined in the shoot during shoot die-back but *P* increased in the root. Additionally, the lateral bud dormancy generally observed in this species during shoot die-back corresponds to declined levels of *P* that is due to the low concentration of N, the greatest effect of which occurs at leaf yellowing particularly in the foliage.

Shoot die-back is preceded by chlorosis in which declines in protein synthesis, synthesis of chlorophyll and protoplasm are expected and may result in the decline of *N* and Mg concentration due partly to withdrawal of these elements. The withdrawal of *N* and *K* from the foliage before leaf abscission is common and is reflected in the lower concentration of these nutrients in older leaves compared to younger ones. This is quite apparent when the mean foliar concentration of the macroelements *N, P, K,* and *Mg* in older leaves, at leaf yellowing, are contrasted to those of younger leaves, at leaf flush (appendix Table 2.3.4).

2.3.3.2 Seasonal and plant tissue changes in concentrations of micro-nutrients of *P. angolensis* seedlings

Seasonal *Mn* concentrations in the foliage were not significant (appendix Table 2.3.2). The concentrations of *Fe* and *B* in the stem and root, *Cu* in the stem and *Zn* in both the foliage and stem varied significantly over the four seasons (appendix Table 2.3.2). *Mn* concentration was significantly different between the foliage and the rest of the tissues (appendix Table 2.3.3) particularly at leaf flush where the largest decline occurred in the root and stem (Fig. 2.3.2). The highest decline in all tissues was during leaf yellowing. Phenophasic changes in mean stem *Fe* and *Cu* concentrations were observed to be lower at leaf flush and the highest at leaf expansion for *Cu* whereas the minimal concentrations for *Fe* occurred during leaf expansion to shoot die-back (appendix Table 2.3.4). The root concentration of *Fe* was highest at leaf flush and not significantly different amongst the rest of the growth seasons. The pattern of *Zn* and *B* concentrations over the four phenological phases did not significantly change (appendix Table 2.3.3). Root *Zn* levels significantly declined at leaf expansion and yellowing but later increased during shoot die-back (appendix Table 2.3.4). Due to its active role in the formation of some enzymes, maintenance of the integrity of ribosomes and as an activator or inhibitor of enzyme systems (Trebst, 1994), *Mn* increases in the root during leaf flush probably for the purpose of increasing the volume of enzymes synthesised in preparation for the initiation of leaf growth.

The higher content of *Mn* in the foliage may be related to its requirement in enzyme and chlorophyll synthesis as well as the involvement of Mn^{4+}, Mn^{2+} and Mn^{3+} in photosynthetic oxygen evolution (Campbell, 1993; Taiz and Zeiger, 1998). The decline in foliar *Fe* content during leaf yellowing occurs in the same pattern as the decline in stem and root *Fe* content. The characteristic pattern of root *Fe* concentration (Fig. 2.3.2) which is similar to that of root *Mn* concentration, reveals a reduced relative decline from leaf chlorosis to shoot die-back. The relative increase in foliar *Fe* content drops slightly at leaf flush after peaking during shoot die-back. The decline in the relative foliar *Fe* content at leaf flush may be due to the fact that *Fe* is a relatively immobile element in the plant (Helmisaari, 1992).

Iron is not readily available in sufficient quantities in the relatively fast growing leaves because of its tendency to precipitate that subsequently reduces the mobilization of the metal to the phloem for long distance translocation. The high foliar *Fe* content during shoot die-back could be ascribed to the tendency of iron to form insoluble compounds that are immobile and are retained in the older chlorotic leaves. Subsequently, the young developing leaves contain less *Fe* during leaf flush compared to the *Fe* content of older leaves during shoot die-back due to the immobility of the element. *P. angolensis* seedlings are very sensitive to *Fe* availability thus Munyanziza and Oldeman (1995) recommended *Fe* fertilization of seedlings preferably with foliar sprays to partly overcome the problem of *Fe* immobility.

2.3.3.3 Seasonal patterns in mineral nutrient concentration

The seasonal concentrations, as depicted in Figure 2.3.3, of *N* and *B* during leaf expansion and shoot die-back, *N* and *Mn* from leaf yellowing to shoot die-back, *K* and *P* from leaf expansion to yellowing, *K* and *Cu* during leaf expansion and shoot die-back, *Mn* and *B* from leaf yellowing to shoot die-back, and *Ca* and *B* during leaf expansion and yellowing showed significant correlations (appendix Table 2.3.6).

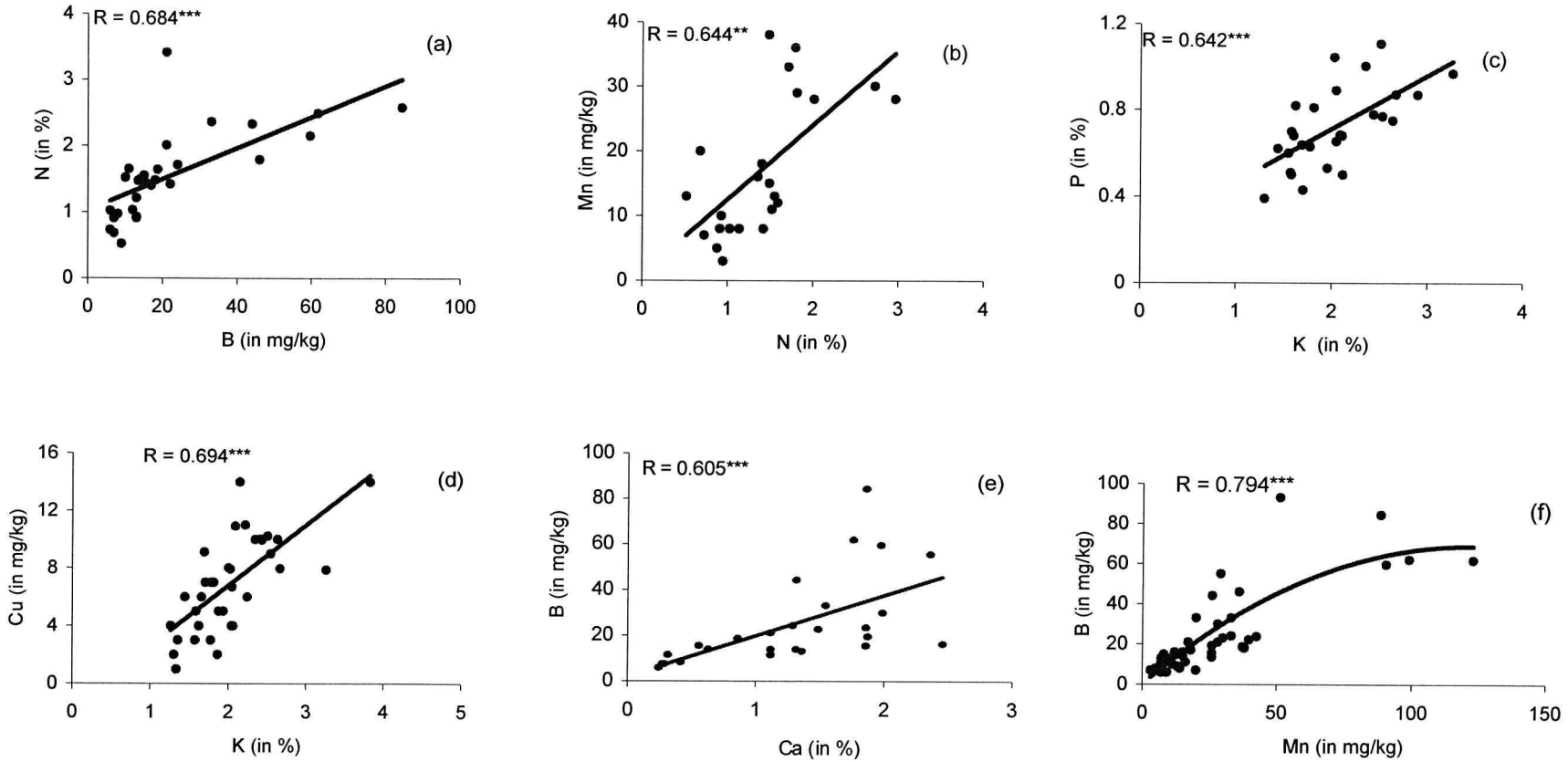

Figure 2.3.3. Relationships between mineral nutrient concentrations, and the seasons for which each relationship is made i.e. (a)=leaf expansion and shoot die-back, (b)=leaf yellowing and shoot die-back, (c)=leaf expansion and yellowing, (d)=leaf expansion and shoot die-back, (e)=leaf expansion and yellowing, and (f)=leaf expansion, yellowing and shoot die-back. Superscripts on correlations indicate significance at the following levels: (**) at p=1% and (***) at p=0.1%.

The decline in *N* and *B* occurs from leaf flush to yellowing but the largest decline is that occurring between leaf flush and shoot die-back in *N*, a 33% decline, and the 29% decline in *B* between leaf flush and leaf yellowing (appendix Table 2.3.3). The decline in *N* and *B* occur during leaf yellowing and shoot die-back. A significant decline which is common to *P*, *Mn*, *Cu* and *B* occur during leaf yellowing whereas *N* and *K* decline from leaf flush to shoot die-back without an increase in concentration after leaf flush. A unique feature is obtained in *Ca* which increases from leaf flush to shoot die-back.

Declines in mineral nutrient concentration during the season of leaf yellowing, with the exception of *Ca*, may be due to the fact that the species physiologically prepares for shoot die-back by shedding leaves.

2.3.4 Conclusion

A consistent pattern revealed significant changes in foliar *N*, *Ca* and *Mg* and stem *Fe* concentrations in relation to phenophases. Significant changes were observed in root *P, K, Ca, Mg, Cu,* and *B*. The mineral nutrient patterns observed in the foliage and roots but less in the stem, may be related to nutrient remobilisation during shoot die-back. Larger relative changes obtained during leaf yellowing and shoot die-back could be an indication that significant volumes of mobile nutrients are removed from senescing tissues. The pattern of nutrient distribution has been observed by Munson and Timmer (1990) and Nambiar and Fife (1991) to be determined by internal controls and relative sink strength associated with developing tissues that are modified by the interaction of environmental variables with nutrient requirements determined by genotype. The general and extensive leaf chlorosis observed in *P. angolensis* occurs towards shoot die-back, which occurs just before the winter season. Sometimes chlorosis overlaps into the shoot die-back season and thus occurs from late summer into early winter or the whole of winter.

Since chlorosis is most often associated with the lack of *N* and deficiencies of *Fe, Mn, Mg, K* and other mineral nutrients (Bidwell, 1979; Van den Driessche, 1984; Salisbury and Ross, 1985; Drechsel and Zech, 1993), it cannot be ascribed specifically to environmental factors as the single cause.

Environmental factors may act as stimuli to activate various physiological mechanisms that affect the relative concentration of nutrients in each plant organ. Some of these mechanisms include the release of and changes in phytohormone concentration. The case for mineral nutrient remobilisation from leaf yellowing to shoot die-back, can be observed by the increase in the mean root concentration of all the mineral nutrients during shoot die-back with the exception of *K*.

Since no foliage develops on naturally regenerated seedlings during winter, the mineral nutrient profile of the species for the root in particular may not necessarily be comparable to that of seedlings growing under a partially controlled environment. Therefore, the presence of leaves on seedlings during shoot die-back probably has a confounding effect on the relative concentration of some mineral nutrients. The decline in foliar and stem nutrient concentration obtained in the season of leaf yellowing in seedlings whose leaves remain on the plant in winter seems to be temporary rather than a process leading to permanent discontinuation of growth in these organs. Leaves remaining on seedlings, which do not fully respond to processes leading to leaf shedding, act as a signal to such processes to downsize the effects of leaf chlorosis, abscission and shoot die-back. Therefore during the period of shoot die-back, *P. angolensis* seedlings are able to tolerate the decline in nutrient concentration either through internal reserves or through the adjustment of plant metabolism that may involve either concentration of reserves in actively growing tissues or redistribution of nutrients.

Due to a decline in stem growth and leaf expansion during winter, *P. angolensis* seedlings significantly scale down the need for the synthesis of cellular constituents as the season of leaf shedding and shoot die-back approaches. This may have resulted in the patterns of mineral nutrient changes associated with phenophases in *P. angolensis* seedlings.

2.4 THE EFFECT OF SEEDLING AGE AT PLANTING AND OF SEED ORIGIN ON SHOOT DIE-BACK IN FIELD GROWN *Pterocarpus angolensis* SEEDLINGS

2.4.1 Introduction

Pterocarpus angolensis DC, a member of the *Fabaceae* family (syn. *Papilionaceae*) which is a subfamily of *Leguminosae*, occurs in Angola, DR Congo, Malawi, Mozambique, Namibia, Tanzania, South Africa, Zambia and Zimbabwe (Boaler, 1966; Vermeulen, 1990; Musokonyi, 1998). The species is considered to be one of the best known and valuable hardwood tree species in eastern, central and tropical southern Africa (Coates Palgrave, 1981; Vermeulen, 1990; van Daalen *et al.*, 1992; Stahle *et al.,* 1999). The wood is easy to work and is therefore used in carvings, furniture, joinery, veneer and as a general purpose timber (Coates Palgrave, 1983; Vermeulen, 1990; Stahle *et al.,* 1999). The value of the species, which includes medicinal uses, has made it one of the most sought after tropical hardwood species by local communities and commercial logging companies. *P. angolensis* is considered an endangered species due to poor stewardship and long-term adverse climatic conditions (Munyanziza and Oldeman, 1995; Musokonyi, 1998 and Stahle *et al.*, 1999). Problems related to stewardship include inappropriate inventory procedures (Stahle *et al.*, 1999), unknown natural regeneration dynamics, and non-existent and inappropriate silvicultural technology (Munyanziza and Oldeman, 1995) and forest management methods specific to ecosystems in which the species occurs.

Natural regeneration of the species in the miombo woodlands is greatly affected by stressful environmental conditions such as the long dry season, annual fires and in some areas by browsing (Von Breitenbach, 1973; Munyanziza and Oldeman, 1995; Stahle *et al.,* 1999). The moisture conserved by the pods during the dry season attracts termites that crack the hull (Graz, 1996) and severe fires occurring in the dry season burn or scorch the pod through which the germinating seed easily breaks during the rainy season.

P. angolensis is sensitive to overshadowing, therefore high natural regeneration rates are apparent mainly in open woodlands or open areas of closed forests, particularly in high rainfall areas. Grazing and browsing have been widely assumed to have a negative impact on the ability of the species to regenerate. The condition that is generally termed "*suffrutex*" (Boaler, 1966; Vermeulen, 1990) in which the shoot dies back to about 3 cm below ground each dry season has been reported and assumed to be the major hindrance to successful regeneration and ageing of *P. angolensis*. Research in *P. angolensis* has been carried out in seed germination at nursery level (van Daalen, 1981; Kasumu, 1998; Musokonyi, 1998), genetic variation at seedling level (Munthali, 1999) and root pruning and fertilisation in the nursery (Munyanziza and Oldeman, 1995; Munyanziza et al., 1998). *P. angolensis* ecology (Boaler, 1966), population dynamics in permanent sample plots (for stem counts, height and diameter at breast height), fire ecology and land use (Graz, 1996) as well as management implications of annual growth rings (Stahle et al., 1999) have also been reported.

Empirical research dealing with the establishment and management of field trials has not been reported so far even though woodlots of the species have been established from seed at Katombora (near Livingstone in Zambia). In order to verify the occurrence of shoot die-back, seedlings of different ages including direct sowing, from Namibia, South Africa and Tanzania were planted near Nelspruit at Sudwala Caves (25° 23' S, 3° 41').

The field trial was established to determine the effect of seed sources and the age of planting stock, including direct seeding, on the occurrence of shoot die-back under field conditions. The assumption was made that the occurrence of shoot die-back are not significantly influenced by seed origin nor the age of planting stock.

2.4.2 Materials and Methods

Seedlings from four seed sources from Hamoye (17° 56' S, 20° 01' E) in Namibia, Iringa (7° 46' S, 35° 41' E) Tanzania, Nelspruit (25° 30' S, 30° 58' E) in South Africa and Kasama (10° 13' S, 31° 12' E) in Zambia, were raised at Ngodwana nursery near Nelspruit. The seedlings were made up of four (4) age classes from 1 to 3 months, with the direct sown seeds making up the 0 month age class.

Seedlings were raised in 80mm x 50mm x 300mm (1.2 *l*) black plastic containers on a concrete floor in Ngodwana nursery. Ngodwana nursery is covered with a 50% shade net. The seedlings for this study were grown in an area of the nursery covered with fibre carbon. Therefore, apart from the roofing all other environmental variables were not controlled in the nursery. Seed was sown in a potting mixture made of two parts woodland soil and one part of fine pine bark. The woodland soil was collected from the Sudwala Caves area where the trial was later established. The potting and establishment site's soil physical and chemical characteristics are in appendix Table 2.4.1. Sowing in the nursery took place in August, September and October for each of the age classes.

Since the Zambian seed source did not have seedlings to plant in all replications, only the Namibian, Tanzanian and South African seed sources were used in this experiment. Grading of seedlings was carried out based on healthy and size (Table 2.4.1). Planting spots, 1.5 m x 1.5 m espacement, were prepared by digging a hole of about 40 cm deep and 30 cm across at the surface. Water retention crystals at a concentration of 5g/*l*, were added to tap water and allowed to dissolve and form a gel for 30 minutes which was then applied at 1 *l* per plant. A third of the gel was mixed with part of the soil from the planting hole and placed at the bottom of the hole. The remainder was also thoroughly mixed with soil and placed around the plant with untreated soil filling the sides. No water was applied to the plants before and after planting.

The experimental design comprised
Replications = 17
Seed sources = 3
Seedling ages = 4 (the fourth is from direct sowing)
Number of seedlings per seed source = 68
Plot size = 1 seedling

Fourteen replications were completed on 19 December 2000 and the remaining replicates were not completed due to lack of time. A survival count was conducted on 5 January 2001 in which mortality was calculated at 13.7%.

Beating up was carried out and the remaining replicates were planted on 5th January 2001. Replicate 7, 14 and 17 from Block 4 (appendix Table 2.4.2) are not part of this report due to the fact that they were not completed due to high mortality in remaining seedlings during the January 2001 beating up. The general model for the analysis was $Y_{ijk} = \mu + \lambda_i + \delta_j + \beta_k + \varepsilon_{ijk}$ where Y_{ijk} is the response obtained at the i^{th} of seed source, j^{th} level of seedling age and in the k^{th} replicate; μ is the mean of the population, λ_i is an effect due to the $_i^{th}$ seed source, δ_j is an effect due to the j^{th} seedling age and β_k is the effect due to the k^{th} replicate.

ε_{ijk} is a random error term associated with all experimental factors and is assumed to be randomly distributed $[N(0, \sigma_p)]$ and independent. Statistical calculations for significance tests and mean comparisons were analysed in SAS Enterprise Guide Release 1.3 (SAS Institute, 2001).

Assessments for the experiment were seedling survival in January 2002, shoot height, occurrence and type of shoot die-back in June 2001 and August 2002. A seedling was classified as having survived when a 'live' stem or shoot was present. Mortality was taken as the number of planting spots without visible signs of either a live or dead plant during the growth season. At the end of the experiment 5 seedlings from each seed source and seedling age were to be randomly dug up from the whole experiment for the purpose of comparing the root structure, top of root diameter, the length of the root tuber as well as the number of roots branching from the root tuber. Due to poor field survival and shoot die-back, only plants grown from the South African seed source were dug up for root examination in August 2002, because it had a complete sample representing all four seedling ages.

2.4.3 Results and Discussion

Results are presented in the form of graphs, tables and photographic images whilst the analysis of variance and the comparison of means are tabulated in Appendices 2.4.3 to 2.4.5. Since shoot die-back did not occur in June 2001 nor January 2002 due to the prolonged rainy season, shoot die-back is only considered for the August 2002 assessment.

Seedling survival and height growth

The age of nursery stock at planting significantly influenced seedling survival (Appendix 2.4.3a). A similar effect is obtained in the age of seedlings growing under natural conditions (appendix Table 2.4.4). Seed sources did not significantly affect field seedling survival in June 2001 or January 2002 (appendix Table 2.4.3b). Despite the fact that the South African seed source was locally obtained, it did not survive or grow better than the Namibian and Tanzanian seed sources. Generally, seedling survival declined in January 2002 in all seedling ages compared to what was obtained in July 2001 (Figure 2.4.1).

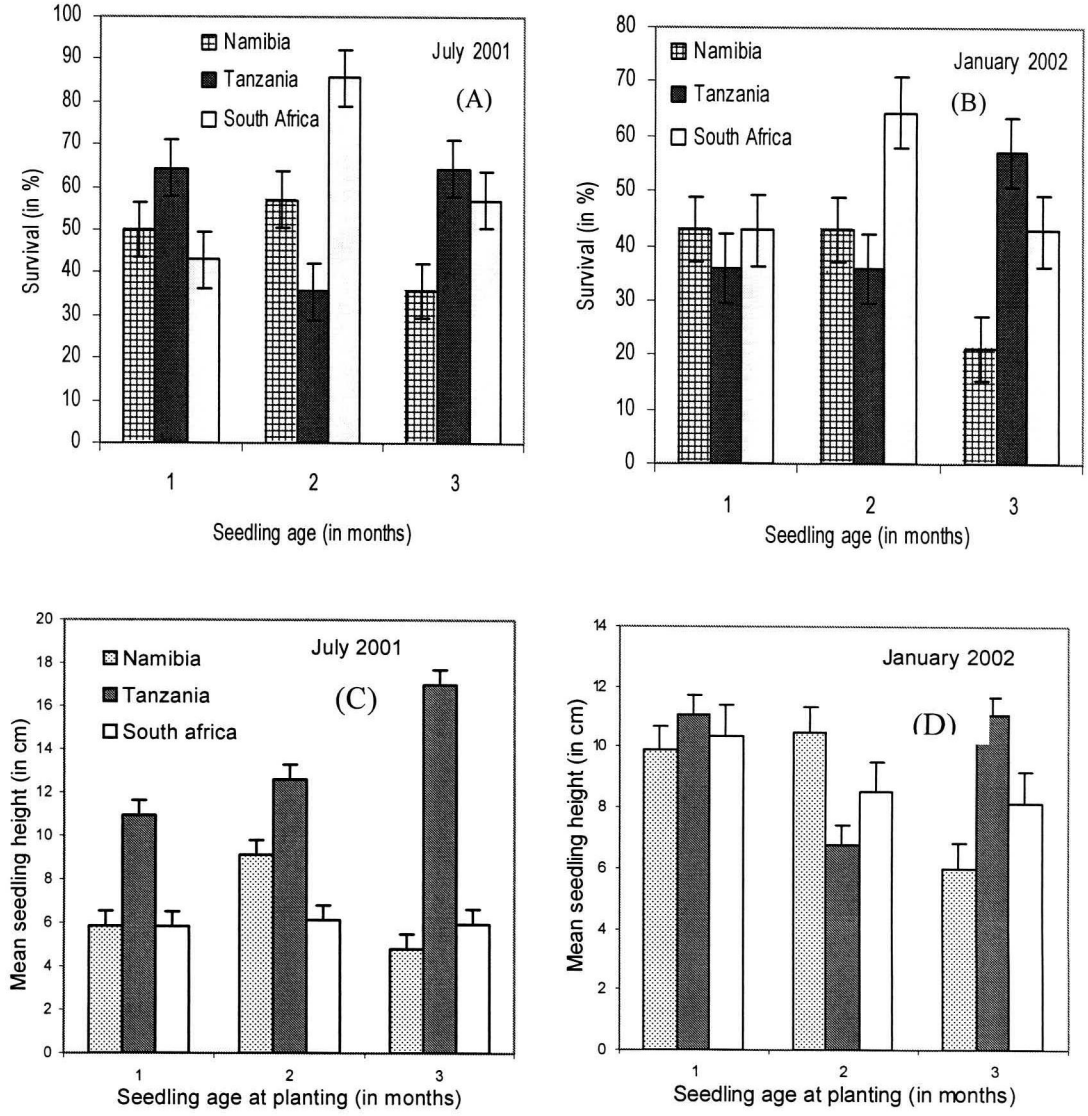

Figure 2.4.1. Seed source differences in the survival [(A) and (B)] and height [(C) and (D)] of field grown P. angolensis seedlings assessed in January 2001 and June 2002. Direct sowing is not indicated since only 1 seedling was recorded. Vertical bars represent the standard error of the unadjusted mean.

As has been observed earlier during the assessment of *P. angolensis* seasonal biomass investment, (cf. Chap. 2, Part 2.1), young seedlings rapidly develop a deep taproot which is succulent and non-woody. This stage of root development is amenable to damage if not properly handled.

Secondly, due to the fact that field conditions are generally harsher than nursery conditions, acclimatisation of young and tender plant parts is long and difficult unless conditions are favourable. Poorly developed storage tissues in the young seedlings could have made the seedlings extremely susceptible to water and nutrient variability under field conditions with low soil moisture. Three month old seedlings may have performed poorer than two month old seedlings due to the fact that stem and leaf growth had almost peaked for the season. Therefore, the vigour required for growth under harsher conditions was reduced. Reaction mechanisms of *P. angolensis* seedlings to water availability is usually through a rapid chlorosis and shedding of leaves. Unless this is accompanied by an increase in moisture, gradual shoot death will occur. This is probably the reason why seedling survival in three months old seedlings is lower than that for 2 month old seedlings (appendix Table 2.4.3b). A higher proportion of three month old seedlings experienced die-back than in the one and two month old seedlings (Fig. 2.4.2).

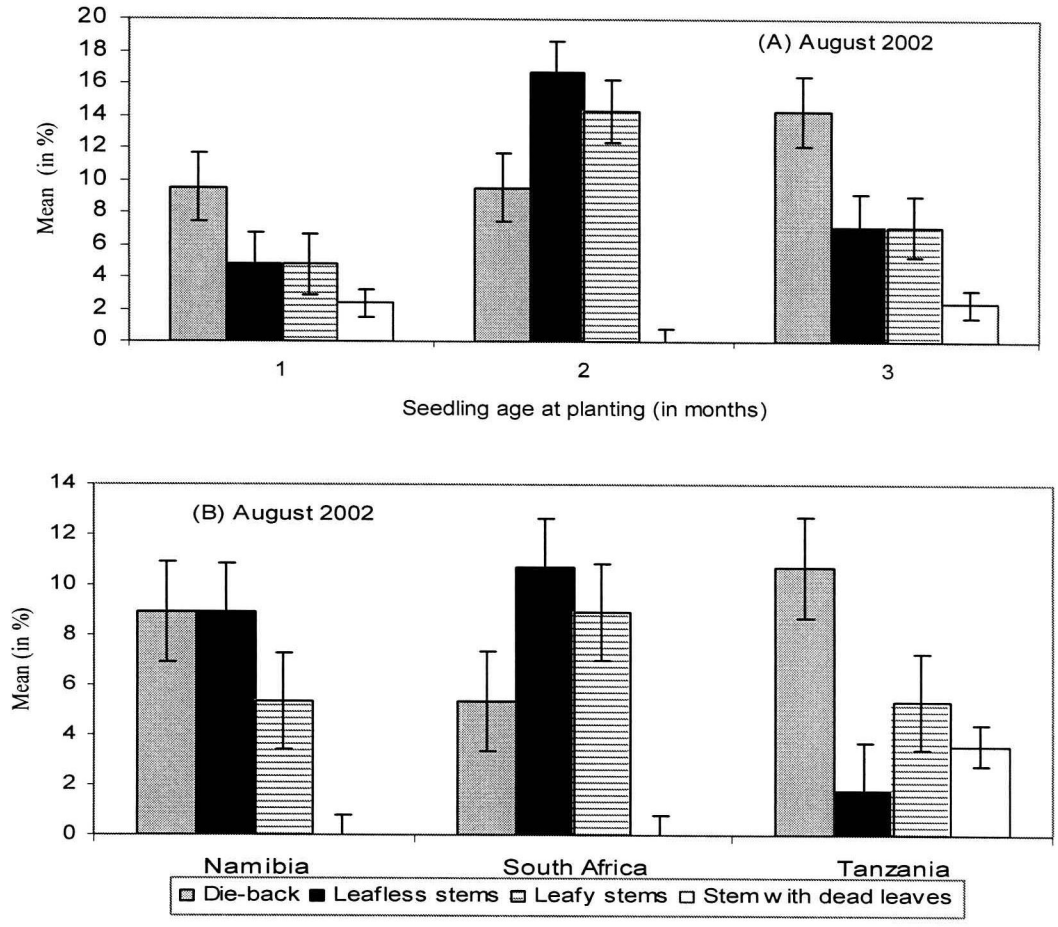

Figure 2.4.2. Trends of shoot die-back and stages of leaf phenology in 20 month old seedlings of P. angolensis assessed in August 2002. Mortality values are arcsine transformations of mortality counts.

Seedling survival from direct sowing was extremely low, almost negligible, partly due to poor germination and unknown optimum field conditions suited to germination of the seed. Several endogenous and environmental factors can be responsible for the low survival, among which seed viability and soil moisture are critical.

Additionally, browsing was evidenced (Fig. 2.4.3) as has been observed in the Zambezi teak forests by Von Breitenbach (1973) who indicates that the fleshy taproot is dug up by wild pigs whereas leaves are browsed by Kudu and other large mammals. Only one seedling was obtained from direct sown seeds in January 2001 and none in June of the same year. Under nursery conditions, seed germination is obtained within the first 7 days due to favourable conditions.

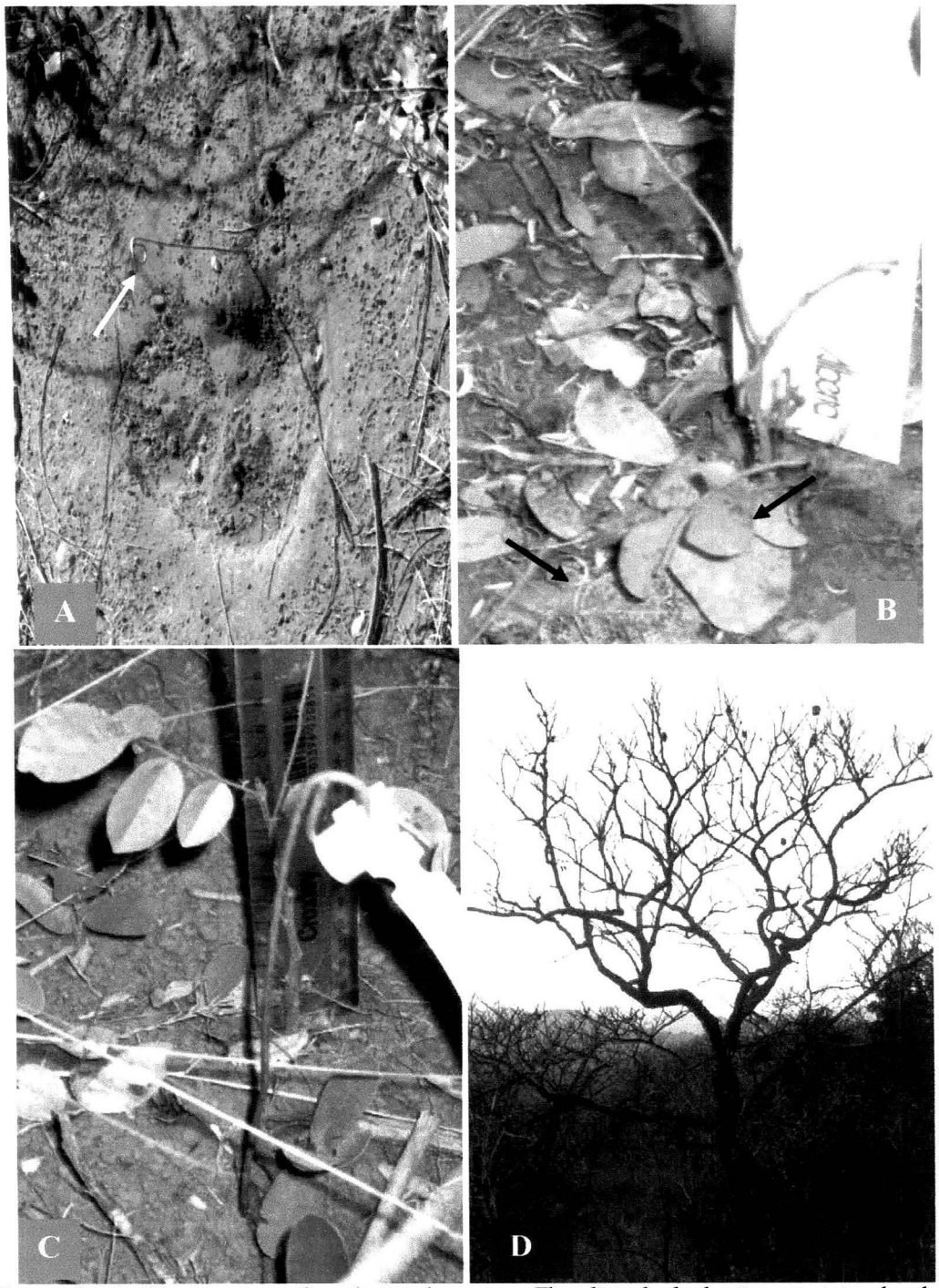

Figure 2.4.3. Planting spot disturbance by game. The disturbed planting spot is clearly delineated from the undisturbed surroundings (A), arrow points to planting spot wire peg. Leafless stems (B) with dry leaves on the ground (arrow) obtained in August 2002 which contrasts to the seedling obtained in June 2001 (C) prior to shoot die-back. The leafless P. angolensis tree (D) in the environment of the field trial is identified by the few out of season fruits in August.

The fact that field inspections were not carried out periodically for the first 2 months may be partly the reason why germination was not noticed until June 2001. Additionally, naturally shed seeds are exposed to the earliest rains that occur from about November whereas direct seeding in this case took place close to the third week of December. The decline in seedling survival, post-June 2001, to 23.2% in August 2002 is not necessarily indicative of the inability of nursery raised planting stock to survive and flourish under field conditions. Post-shoot die-back recovery levels play a major role in the survival mechanisms of the species. This may be assumed to be correlated with the extent of development of the root system of the species and less with how large the photosynthetic surface is.

Height growth assessed at 7 months of field growth showed variability amongst the seed sources (appendix Table 2.4.3) and not amongst seedling ages at planting. Heights recorded in August 2002 were only made on live shoots and not those that had experienced shoot die-back, and are therefore not included in this discussion.

The study did not take into account the effect of the height of planting stock on subsequent height growth due partly to the fact that shoots last only for the growing season and may have no correlation to newly regenerated shoots. It is apparent in Table 2.4.1 that two month old nursery seedlings and the Tanzanian seed source had taller seedlings.

Table 2.4.1. Mean heights of seedlings at field planting and over 13 months of field growth

SEED SOURCE	MEAN HEIGHTS (in cm)							
	Seedling height at field planting				*Seedling height at 13 months of age*			
	1 month*	2 months	3 months	**Seed source means**	1 month	2 months	3 months	**Seed source means**
Namibia	6.95	9.78	7.50	**8.21** (0.57)	9.88	10.50	6.00	**9.35** (0.82)
South Africa	6.53	10.90	6.43	**7.11** (0.97)	10.38	8.50	8.17	**8.94** (0.99)
Tanzania	10.44	14.65	16.28	**13.32** (0.89)	11.10	6.78	11.04	**9.87** (0.65)
Seedling age means	**8.61** (0.74)	**11.84** (1.10)	**10.89** (1.46)		**10.42** (1.01)	**8.67** (0.70)	**9.14** (0.87)	

* *indicates age of seedling at field planting. Numbers in parentheses are standard errors.*

2.4.3.2. Occurrence of shoot die-back in seedlings growing under field conditions

Shoot die-back occurred uniformly amongst the seed sources and age of planting stock (appendix Table 2.4.5a) even though live-leafless stems and seedling mortality varied amongst the ages of planting stock. Two month old seedlings had comparatively more seedlings in August 2002 than the other two ages as well as more plants without leaves. The presence of a high proportion of leafy than leafless plants, particularly in the South African seed source, is an indication of the fact that the seed source is local and adapted to conditions under which it was tested. August is late in the season of shoot die-back and it would be expected that a higher proportion of plants would be without leaves by then.

Table 2.4.2. Mean per cent of *P. angolensis* seedlings exhibiting shoot characteristics associated with shoot die-back

SHOOT CHARACTERISTICS	SEED SOURCE			SEEDLING AGE AT FIELD PLANTING (in months)		
	Namibia	*S. Africa*	*Tanzania*	*1*	*2*	*3*
Shoot die-back	8.93 (3.85)	5.36 (3.04)	10.71 (4.17)	9.52 (4.58)	9.52 (4.58)	14.29 (5.46)
Live-leafless stems	8.93 (3.85)	10.71 (4.17)	1.79	4.76 (3.33)	16.67 (5.82)	7.14 (4.02)
Leafy stems	5.36 (3.04)	8.93 (3.85)	5.36 (3.04)	4.76 (3.33)	14.29 (5.46)	7.14 (4.02)
Live stem with dead leaves	0.00	0.00	3.57 (2.50)	2.38	0.00	2.38
Mortality	76.79 (5.69)	75.00 (5.84)	78.57 (5.53)	78.57 (6.41)	59.52 (7.67)	69.05 (7.22)

Numbers in parentheses are standard errors of unadjusted means and mean per cent not accompanied by a standard error indicates data from a single observation. Therefore these are not classified as means.

Secondly, the fact that sporadic rains were being obtained by then (data not available) may have affected the progression of shoot die-back. Also, leafless stems are an indication, in this species, of the onset of progressive events leading to shoot die-back as long as it takes place in the dry season prior to the first rains. At this time of the year, no correlative inhibition (within season dormancy) was suspected neither has it been observed under nursery conditions. Similar to correlative inhibition are events taking place in seedlings that had not completed the shoot senescence sequence by spring.

Shoot senescence under field conditions has similar patterns to the nursery phenomenon, the difference being in the final pattern of die-back as well as the number of seedlings affected. Die-back starts with leaf loss much earlier in summer, sometimes as early as March or a few weeks after the summer rains.

Leaf loss is not followed immediately by progressive shoot die-back, but leafless stems can be found as late as August, not only in planted seedlings but in naturally regenerated seedlings too. Whether this trend is due to the fact that the plant must complete nutrient translocation gradually and over a long period of time, or is a way of not completing shoot die-back early in anticipation of environmental changes, is not clear.

It can be assumed though that shoot senescence is an ordered and very gradual process, under natural conditions, in which the plant deliberately monitors the gradual changes in both the environment around the shoot and the root. Generally *P. angolensis* shoots of seedlings have finite functional lives that are restricted to between the first rains and June, with temporal separation governed by the length of the rainy season. Although the life of seasonal organs may be extended, in the end they have to be renewed in order for the plant to have functional assimilatory organs in the next growth phase (Noodén, 1988). The senescence signal in *P. angolensis* has two stages: it is first exerted upon the oldest leaves located at lower part of the stem, there exists very little difference within each compound leaf as to which pinnae is first affected; the signal is then transmitted through the leaf from the shoot apex downwards. Therefore senescence in this species is preceded by cessation of bud initiation and leaf production is followed by leaf yellowing.

The age which is associated with the definition of senescence (Silvertown and Charlesworth, 2001) in this species, should refer to the age-within-season and not the seedling age especially when an increase in mortality rate with age is considered in the context of *P. angolensis*. Leaf yellowing in *P. angolensis* may also occur throughout the season of rapid growth but the difference is in the existence of continued bud break and leaf production. Leaf yellowing that leads to or signals the early stages of shoot die-back differs from the within-growth-season leaf yellowing by the pattern of pinnae yellowing.

Leaf yellowing leading to shoot die-back occurs uniformly over the whole pinnae and is not only in the inter-veinal regions of the pinnae. Secondly, complete leaf loss may not also be indicative of shoot die-back but depends also on the season when it occurs and whether bud break ceases too. The cessation of bud and leaf production does not necessarily indicate shoot senescence, unless the shoot starts to die and dry up from the shoot apex downwards. This may also be incomplete as has been observed under nursery conditions, but once initiated in the right season of field conditions it is irreversible. Shoot senescence in this species is closely associated or occurs during the dry season prior to the hot season. The trough, or period of low precipitation in Figure 2.4.4, is the period in which senescence progressively manifests itself.

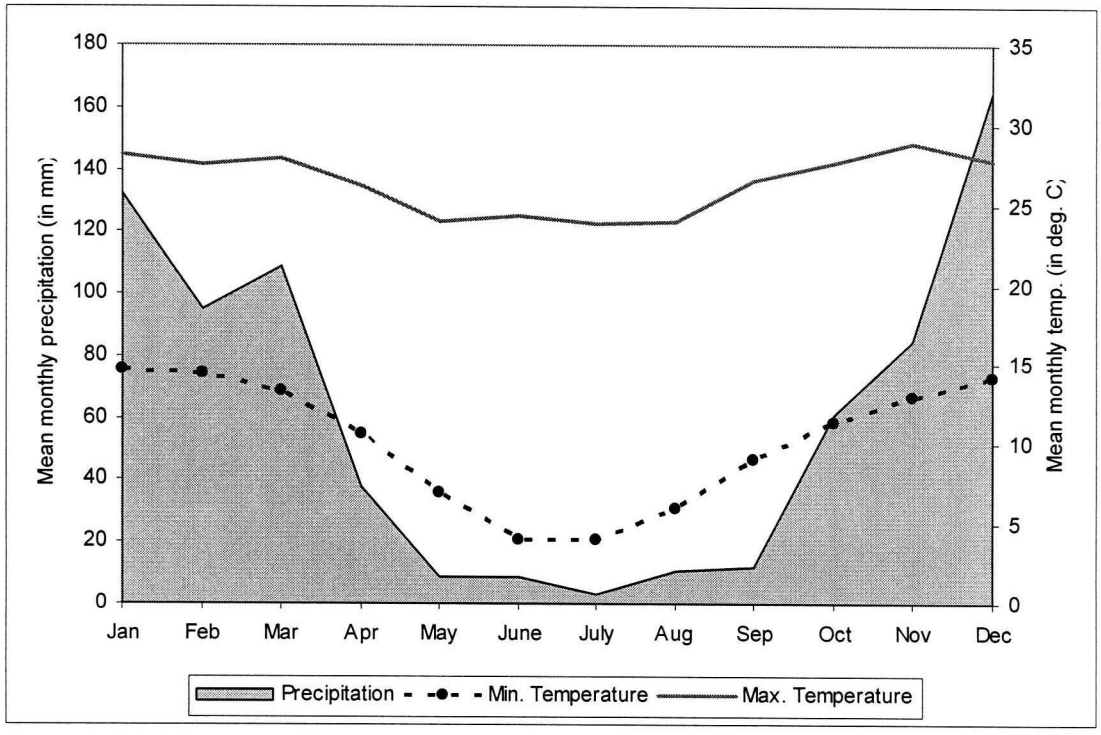

Figure 2.4.4. Mean monthly temperature and precipitation for P. angolensis habitat in South Africa. Data obtained from Computer Centre for Water Research at the University of Natal. Climatic data from stations located within 24° 05' – 25° 58' S and 30° 05' – 31° 50' E recorded between 1920 and 1998 was averaged from 20 years selected randomly from this data. Mean maximum temperature data is only for 1990 and 1991.

The cyclic changes in environmental conditions in which seasons favourable for growth are separated by periods when growth is slow or entirely suspended (Villiers, 1975), create an evolutionary change in which *P. angolensis* has evolved to resist these changes by synchronizing its cycles of life processes with changes in optimal temperature and precipitation.

Changes in life processes are mainly apparent in the above ground structures even though declined, and not suspended growth, is still obtained in the root under nursery conditions (cf. Chap.2, part 2.1). Whether root biomass investment continues during the period of shoot die-back or is suspended under field conditions is not known but can only be inferred from observations made under nursery conditions in an environment in which the mean monthly temperature is more extreme than in the natural habitat of the species. Therefore, the main phenological traits of plants in the Zambezian plateaux(May-June = cold dry season; August-September = hot dry season; October-November = early rainy season; December-February = rainy season; March-April = late rainy season) are influenced by rainfall and temperature (Menaut et al., 1995).

The gradual progression of shoot senescence, under field conditions, may be related to the gradual decline in the level of the water table. As the water table recedes, the moist front of the soil profile gradually recedes too thus creating a declined moist root zone environment. Plants easily sense this development and send positive signals to the rest of the plant that increases in intensity with increasing drought conditions in the root zone. Retardation of shoot growth has been observed to occur under moderate rates of water table decline even though root elongation is enhanced thus indicating the fact that overall reallocation of growth processes takes place (Pereira, 1994; Rood *et al.*, 2000). Following the water table decline, roots tend to react by extension growth into water containing soil compartments to enhance continued growth (Lösch, 1989; Lösch and Schulze, 1994; Taiz and Zeiger, 1998).

A reduction by 80% in soil extractable water results in the dimunition of maximal leaf conductances and assimilation values. Since declined soil water in *P. angolensis* habitats starts when plants have already developed a maximal leaf area, plants react through leaf senescence and leaf fall. Leaf area adjustment is a vital long-term mechanism that enhances the plant's fitness for a water limited environment (Taiz and Zeiger, 1998). This might be the reason why live leafless shoots of *P. angolensis* remain for quite some time after all the leaves have been shed off. Effects of changes in water availability affecting leaf growth and leaf area may become manifested at comparatively less declined levels of soil extractable water.

Other metabolic pathways and reactions are affected one after the other whenever a new threshold value of soil extractable water is reached (Hsiao, 1973; Richter and Wagner, 1983). Additionally, drought-induced growth inhibition can lead to sink limitation and feed back control of photosynthesise (Kaiser, 1987; Wilson *et al.*, 2000). The leaf adjustment decreases carbon and energy consumption, and a larger proportion of assimilates can be translocated to the root where they are involved in continued growth (Taiz and Zeiger, 1998). The resulting effect of this adjustment can be premature leaf senescence under water deficit (Parkinson and Day, 1983) in which old, unproductive leaves are continuously shed (Richter and Wagner, 1983) gradually followed by younger productive leaves. Water availability is therefore an important factor that limits plant productivity and crop yields in diverse ecosystems. Among the many environmental variables, water is one of the most limiting variables for crop production on a global basis (Hsiao *et al.*, 1976). Therefore, biomass investment is directly proportional to water supply and plant water use (Beadle *et al.*, 1993).

Seedlings were dug up only from the South African seed source that had at least a surviving seedling for each age of planting stock. Root examination could not be replicated because of poor survival and the remaining seedlings were to be handed over to the owner of the land on which the experiment was established. The sizes and structure of the root show differences, particularly the length of the tuber (Table 2.4.3).

Table 2.4.3. Root size for the three seedling ages and direct seeding in 20 months old *P. angolensis* under field conditions

SEEDLING AGE AT FIELD PLANTING (in months)	ROOT COLLAR DIAMETER (in cm)	LENGTH OF ROOT TUBER (in cm)	ROOT BRANCHES
1	3.1	9.1	3
2	3.0	20.3	2
3	3.1	16.9	2
Direct seeding	2.8	Uniform taper	none
Mean	3.0 *(1.4)*	15.4*(57.4)*	2

Numbers in parentheses are standard errors.

The seedling from direct seeding had a uniformly tapering root with almost the whole root appearing like a tuber even though it was difficult to dig down to the very tip of the taproot (Fig. 2.4.5).

The uniform taper may be due to the fact that the root was not initially confined to a container prior to its release during field growth, which is in contrast to the tubers obtained in seedlings raised in the nursery. The uniform taper does not only reflect the absence of a container but might be proof of rapid taproot growth in seedlings arising from direct seeding. If this is the case, chances are high that naturally regenerated seedlings may have similar taproot characteristics of a uniform taper in 2 year-old seedlings. Seedlings raised in the nursery in 2.4 m plastic tubes also show a poor formation of the tuber (cf. Chap. 2, part 2.1) and it is likely that tuber formation under field conditions closely follows the longitudinal extension growth of the taproot.

Probably the size of the tuber determines the duration of shoot die-back and cessation of the *suffrutex* phase of growth. The extremely hardened and woody 1-3 cm top portion of the tuber has a very important physiological function: either it evolved due to the occurrence of or as the plant's evolutionary mechanism to protect itself against the effects of shoot senescence or the harsh environmental conditions of the tropical dry season. Fissures may serve to protect the seedling against extreme conditions as well as bush fires that are common in its habitat. A closer examination of Figure 2.4.5a reveals that new shoots originate from the sides of the top part of the root and not directly from the top of the root. This is indicates that no remnant of the previous season's senesced shoot has anything to do with both the origin and regeneration of new shoots. In nursery grown seedlings, it has been observed that in seedlings in which complete shoot senescence occurs, the next shoot growth will always originate from below the soil surface and on the side of the root. In some cases new shoots originate from the top of roots in situations where the previous season's shoot has had a fresh stub still remaining on the root. New shoots therefore originate from adventitious buds that are located within the fissures near the top of the root.

The extremely hard fissures on top of the tuber rarely extend further than 3 cm along the length of the tuber in young seedlings. Fissure development goes with age and the conditions under which the plant is growing. Even under nursery conditions, consistent drying of the topsoil leads to the hardening of the surface closest to the root collar. The development of fissures may also serve to protect adventitious buds against desiccation during the long tropical dry season, in which the tender meristematic tissues are shielded from the harsh environment.

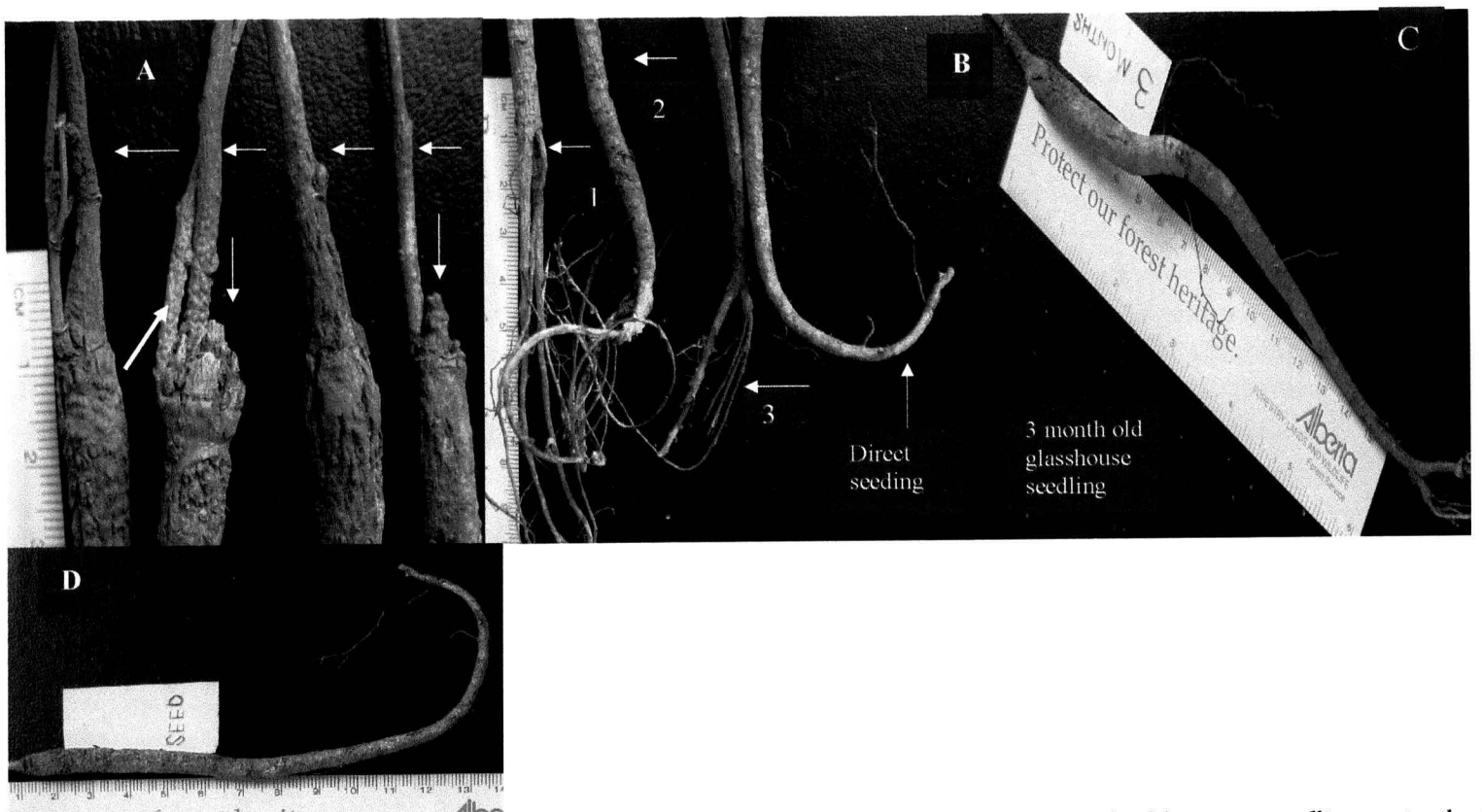

Figure 2.4.5. Field grown 20 months old seedlings' roots showing from left to right in (A) 1-3 month old nursery seedling roots, the fourth is from direct sowing. Horizontal arrows indicate 2 shoots from 1 and 2 month old seedlings whereas the 3 months and direct seeding seedlings have a single stem. Vertical arrows point out the stub remaining after shoot die-back of the previous season's shoot. Notice the fissures occurring only on the 1-3 cm top part of the root. (B) Branching of the taproot obtained only in nursery grown seedlings and absent in direct seeding. The numbers correspond to age in months at field planting. Note the taper in direct seeding compared to the 1 month old nursery seedling (B) which is also apparent in the 3 month old nursery seedling (C) and direct seeding (D).

Secondly, there may be a relationship between the development of hardened tissues on the surface of the top part of the root and the progression of the wet front in the soil during the early days of the rainy season. Meristematic tissues located under the cork cambium may act as detectors of sufficient soil moisture only after water has penetrated these thick layers covering the top part of the root. Therefore, the overall characteristics of the root system of *P. angolensis* hinge not only on the growth of this species but also on the occurrence of shoot die-back.

2.4.4. Conclusion

Pterocarpus angolensis forms very complex ecological and survival relationships with its environment. Browsing by game occurs over the natural range of the species, and is mainly confined to seedlings since no browsing in coppices has been reported. Seedlings suspected to have been browsed never survived, nor were any remains found on the planting spots. Depending on the period between the browse and the field inspection, it may be that the whole plant was dug up. Seedlings planted in the field require special protection such as those used in the Zambezi teak forests (Mwitwa *et al.,* unpublished) was where the seedling was enclosed in mesh wire made into a cone or using twigs to make cones around each seedling. This tends to be expensive, especially when using mesh wire, since it has to be periodically replaced depending on the height growth of each seedling. Additionally, the cones made of twigs have to be removed during the dry season due to the risk of fire unless the planted area is fully fire protected.

Shoot die-back in the species occurs during the dry season prior to the hot part of the dry season and cannot be categorized into different types as is the case under nursery conditions. Complete shoot die-back occurs progressively from the shoot apex downwards and the whole stem right down to the root collar senesces. A short dry stump may remain, which when it occurs, is a fire risk because it can act as an entry port to the remaining part of the plant. In other plants, no stump remains and the next shoot generation arises out of adventitious buds located on the top parts of the root.

The fact that the age of the planting stock and seed sources do not show significant variation amongst themselves may be indicative of common ancestry in the phylogeny of this species. As a survival mechanism, shoot die-back is a form of adaptation that is likely to be common to all patria in the natural range of the species. Finding an area in which shoot die-back does not occur may be most unlikely, even though differences in the timing and length of the occurrence may vary based on relative changes in environmental conditions. It is probable that *P. angolensis* evolved the phenomenon of shoot die-back to ensure biological persistence of subsequent generations by overcoming its failure to persist in its particular environment. The natural patria of *P. angolensis* reflect areas in which it has reached a balance between colonization and extinction which in turn has determined the natural boundary of the species. Shoot die-back in field grown seedlings was not found to be associated with any pathogenic attacks, unless these were undetected with the naked eye. Senescence in other species has been associated with increased activity of pathogenic fungi (Packman *et al.*, 1992).

P. angolensis seedlings for out-planting should be kept under nursery conditions for two months at the most. It has been shown that two month old seedlings have better survival and comparative growth in terms of height than one and three month old seedlings. Chances are that two month old seedlings have an adequately developed root system suitable for enhancing survival and growth. Even though all shoots senesce each year, the size of the above ground plant parts is only important if it has a large and efficient photosynthetic surface. The most important aspect of *P. angolensis* seedlings under field conditions that must be considered is survival and not necessarily shoot growth unless shoot size can directly be related to root size. Shoot die-back in the case of *P. angolensis* may be an evolutionary mechanism through which seedlings cope with drought, fire and other adverse environmental variables for survival during the long tropical dry season. The form of organ senescence taking place is a slow developmental and morphological adaptation and may probably involve other metabolic processes such as changes in the regulation of the hormonal balance of the plant. Therefore, shoot senescence in *P. angolensis* is one of the most visible symptoms of declining soil extractable water during the cool to the warm dry season.

CHAPTER 3

3. WATER TREATMENT EFFECTS ON CHL. a FLUORESCENCE TRAITS OF *Pterocarpus angolensis* SEEDLINGS

3.1 Introduction

Pterocarpus angolensis DC is distributed across central, eastern and southern Africa in habitats that vary widely in moisture supply capacity, from relatively wet areas (1300mm mean annual rainfall) to semi-arid sites (less than 1000mm mean annual rainfall). The species wide distribution across varying habitats that include semi-arid areas suggests the existence of drought tolerance characteristics. However, there is a dearth of information for this species regarding the physiological mechanisms that confer drought tolerance. The suffrutex of the species experiences shoot die-back which is a phenomenon in which the above ground plant parts senesce during the cool part of the tropical dry season. A new shoot is regenerated at the start of the next growing season during the warm and wet parts of the year. The sequence of shoot die-back and regeneration may last up to 10 years before the seedling breaks out of the suffrutex stage. Therefore, manifestation of shoot die-back is strongly associated with low temperature and dry conditions.

Shoot die-back is preceded by leaf chlorosis and death. This trend is closely associated with the decline in precipitation and temperature. Since shoot die-back occurs in the cool and dry part of the year, water and temperature have been strongly assumed to be the major environmental factors involved in the occurrence of shoot die-back. An understanding of the effect of water or temperature (or both) may enhance the understanding of the reasons as to how and why shoot die-back occurs. One of the most rapid and non-invasive screening tests that can be used to assess stress effects on photosynthetic samples is the JIP-test (Strasser, 1988; Strasser *et al.*, 1999).

The JIP-test, based on relationships between primary reactions of photosynthesis and Chl. *a* fluorescence, can be used to measure a large sample within a short time span (Strasser, 1988; Krüger *et al.*, 1997; Strasser *et al.*, 1999).

Chlorophyll *a* fluorescence is a potentially useful indicator of the reactions of photosystem II (PS II) (Weis and Berry, 1987) and represents a rapid and non-invasive screening test (Krüger *et al.*, 1997) in which environmental conditions do not change significantly so as to affect the outcome. The physiological state of a plant changes, such as the photosynthetic system, in order to adapt to changes in environmental conditions. Even though the actual physiological state of a plant at a given moment is partly a function of the states the seedling has gone through, the shape of the associated fluorescence transient is determined by its physiological state at the time assessments are made and by the physical and other conditions surrounding it (van Rensburg *et al.,* 1996; Krüger *et al.*, 1997).

Quanta absorbed by chlorophyll pigments associated with PS II may be used in photochemistry, transferred to photosystem I (PS I), or dissipated as heat or fluorescence (Salisbury and Ross, 1985; Weis and Berry, 1987; Taiz and Zeiger, 1998). *In vivo* chlorophyll fluorescence is emitted only by chlorophyll *a* even though other pigment components such as phycobillins, protochlorophyll, or pyridine nucleotides can be studied by using fluorescence methods (Goedheer, 1972). The relationship between primary reactions of photosynthesis and chlorophyll *a* fluorescence was determined by Kautsky and Hitch in 1931 (Strasser *et al.*, 1999). Upon light excitation of a dark-adapted photosynthetic sample, the chl. *a* fluorescence emission exhibits a fast polyphasic rise to a maximum that is followed by a decline, in a short time span, to a steady level. The rising phase of the chl. *a* fluorescence transient, which reflects the primary reactions of photosynthesis, is unaffected by temperature changes (up to 30^0 C) and the presence of poisons. The declining phase of the chl. *a* fluorescence transient correlates well with increase in CO_2 assimilation.

Variations in chlorophyll fluorescence *in vivo* occurs as a consequence of changes in the redox level of the primary electron acceptor (Q_A) of PS II (photosystem II), or through a nonphotochemical mechanism which seems to be associated with the acidification of the inner thylakoid space, ΔpH, and related conformational changes occurring upon energization of the chloroplast membrane system (Goedheer, 1972; Weis and Berry, 1987).

Environmental factors that include light intensity, CO_2 concentration, water deficit and temperature, which influence photosynthesis, induce changes in both the steady-state quantum yield for net electron transport and changes in the yield of chlorophyll fluorescence. The measurement of chl. *a* fluorescence during photosynthesis can be used to ascertain the quantitative estimates of the effective quantum yield or net electron transport. The fluorescence transient is used as a biosensor to analyse the dynamic capacities of plants to changes in the physical parameters of their environment.

The experiment was aimed at understanding the ability of *P. angolensis* to adapt from dark to light conditions when subjected to different water treatments in order to ascertain the likely effects of water treatments on the photochemical competence of seedlings. Water treatment were assumed to have no significant effects on the ability of *P. angolensis* to adapt from dark to light conditions through the evaluation of the Chl. *a* fluorescence kinetics.

3.2 Materials and Methods

Pterocarpus angolensis seed from Nelspruit (25° 30'S 30° 58'E) was sown at a depth of 2-3 cm in 30 cm deep 1.2 *l* black plastic bags, in sandy-loam soil with a pH of 6.9 (appendix Table 2.2.1) in December 1999. The potting mixture was made up of 2 parts sandy soil mixed with one part of fine compost that was inoculated with soil containing mycorrhiza from a *P. angolensis* site near Nelspruit. Each bag received 70 ml of water per day in summer and 35 ml every other day in winter for 25 months prior to the application of treatments. Water was applied through over-head sprays thrice each day in summer and once in winter.

Ammonium sulphate containing 21% N [210 g/kg N (w/w)] was applied to each seedling at 10 ml of a 2 g/*l* solution on a weekly basis in the first season of growth but the frequency was thereafter reduced to one application per month. Iron chelates (with 6% Fe) were mixed with water at 400 mg/*l* and applied at 1 *l*/10 m^2 of nursery space. Additionally seedlings were raised on a commercial fertilizer, Multifeed® which was applied once per month as a foliar application. In all foliar applications a wetting agent was mixed with the nutrient solution at 1 ml/5 *l*.

Fertiliser applications were only carried out during the summer. Non-nutritional nursery problems with the seedlings occurred with respect to aphids, mites and red spiders which were eliminated by the application of Metasystox R® at 2 ml/*l* for aphids and mites, and cyhexatin 600 SC® at 1 ml/2 *l* for red spiders.

The study was conducted in a covered glasshouse with a uniform water regime but with varying temperature, light and humidity conditions that were influenced by the external environment. The daily temperature and humidity in the nursery fluctuated depending on the prevailing climatic conditions. The maximum and minimum mean monthly temperatures recorded were 38° C and 6°C over the test period. Environmental control in the nursery was limited to cooling by two thermostatically controlled exhaust fans that were set at 35° C.

The conditions in the nursery where this experiment was conducted were as described in 2.1.2. Roofing for the nursery was made of fibre glass that reduced solar radiation by 30%. Solar radiation varied seasonally and diurnally. Seedlings that went through shoot senescence over the first two winter seasons but survived and produced a new shoot the following spring were selected and used in the study. The water holding capacity (WHC) of the soil was ascertained using the volumetric method according to Wilde and Voigt (1955). Therefore, the WHC was partitioned into 5 water treatments, i.e. 30%, 40%, 60%, 80% and 100% of WHC. In January 2002, twenty-five seedlings were sub-divided into 5 blocks of five seedlings each and each treatment applied to one seedling per block. Seedlings were maintained at 80% of the WHC for four days whilst Chl. *a* fluorescence was measured once every day. The five water treatments were applied on the fifth day and fluorescence readings taken from the same pinnae for seventeen days starting from day 5. Therefore, day 1 of treatment represents day 5 of the experiment. The JIP-test (Krüger *et al.*, 1997), based on the relationship between primary reactions of photosynthesis and Chl. *a* fluorescence, was carried out using a PEA (Plant efficiency analyser; Hansatech, UK) instrument with a 10 µs time resolution and a measuring time of 1 s. The actinic light used for light-adaptation was provided by the light source of the instrument (i.e. red light with a peak at 650 nm with a maximum 3000 µmol m^{-2} s^{-1} intensity which is classified as equivalent to 100% actinic light in this study). The two intensities of actinic light used for light-adaptation and excitation, are expressed as percentages of 3000 µmol m^{-2} s^{-1}.

Chl. *a* fluorescence was taken twice, after 20 to 60 minutes of dark-adaptation with leaf clips (Hansatech, UK) and after 100 s of a 3% (80 μmol m^{-2} s^{-1}) actinic light-exposure, at 100% saturation intensity on each pinna every day. Assessments were made between 8 h and 10 h in conditions in which the temperature remained below 30°C. The procedure is illustrated in Figure 3.1 using data from the assessment.

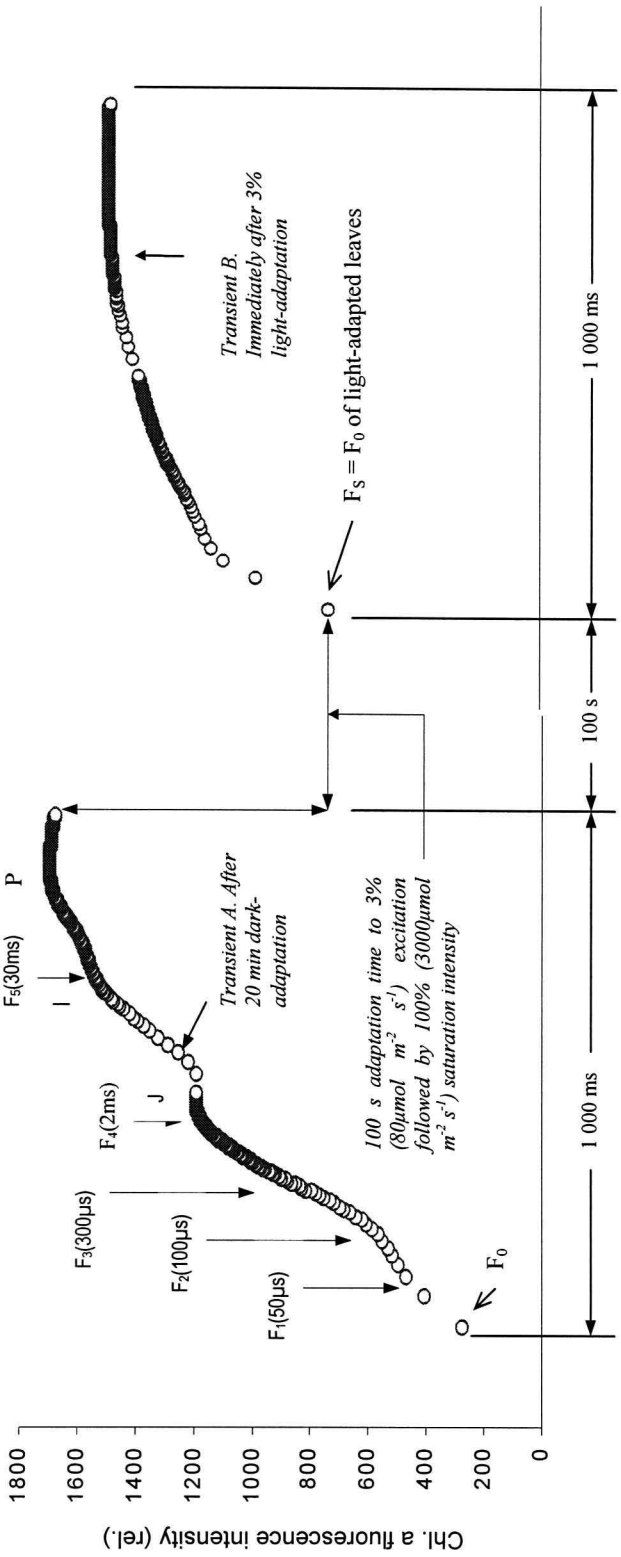

Figure 3.1. Examples of Chl. a fluorescence transients exhibited upon the excitation of (transient A) dark-adapted leaves and (transient B) light-adapted leaves of P. angolensis seedlings. Transients are from averaged data for all water treatments for days 9, 10 and 11. Leaves previously excited with a 3000 µmol m^{-2} s^{-1} (transient A) were light-adapted for 100 s with a 80 µmol m^{-2} s^{-1} excitation followed by a 3000 µmol m^{-2} s^{-1} excitation (transient B). Fluorescence transients are plotted on a logarithmic time scale from 0.01 ms to 1 s, the interval between the two transients of 100 s is not to scale.

Data was captured on the same instrument and later downloaded into Biolyzer (v.3.0.6, Release 25, Bioenergetics Laboratory, Switzerland, Jan 2002) for the calculation of JIP-test parameters. Since a strong light pulse was given to each photosynthetic sample immediately after excitation with 3% (80 μmol m^{-2} s^{-1}) actinic light for 100 s, the initial fluorescence (F_0) taken after light-adaptation was taken to be a measure of the steady state fluorescence signal (F_s). The data was then copied into an MSExcel (Microsoft Corporation Ltd, 1999) worksheet. Statistical calculations for significance tests and mean comparisons as well as Chl. *a* fluorescence transients were carried out in Statistica 6 (StatSoft Corporation, 2001). Prior to the commencement of assessments the minimum dark-adaptation time and light saturation were assessed following the procedure outlined in the PEA operator's manual. The minimum duration of dark-adaptation and light saturation were 16 minutes and 60% of actinic light (1800 μmol m^{-2} s^{-1}) respectively.

Chl. *a* fluorescence transients were plotted using the means of replications for each water treatment for days 9, 10 and 11 only. The two sets of transients from dark- and light-adapted states are as obtained from the PEA. Data used in the statistical analysis are the means of day 9, 10 and 11 of water treatment application. Data for day 11 in replicate 2 of the 40% water treatment, light-adapted samples was excluded from the analysis due to the fact that the transient had a "spike" where the Chl. *a* fluorescence yield at 1.26 ms of 2048 relative units was higher than F_M of 1087 relative units. The transient for this water treatment is excluded from the graphs presented in the results. Statistical analyses and comparison of means are also presented for the difference between the dark- and light-adapted. Formulae used to calculate chl. *a* fluorescence expressions are listed in Table 3.1 according to Strasser *et al.* (1999).

Table 3.1. Summary of the JIP-test formulae using data extracted from the fast fluorescence transient

Extracted and Technical Fluorescence Parameters	
F_0	$= F_{50\mu s}$, fluorescence intensity at 50µs
F_J	= Fluorescence intensity at the J-step (at 2ms)
F_M	= Maximal fluorescence intensity
V_J	$= (F_{2ms}- F_{50\mu s})/(F_M- F_{50\mu s})$
$(dV/dt)_0$ or M_0	$= 4X(F_{300\mu s}- F_{50\mu s})/(F_M- F_{50\mu s})$
S_m	= normalized area { $Area/(F_M-F_{50\mu s})$
N	$= S_m.M_0.(1/V_J)$ turn over number of Q_A
Quantum Efficiencies or Flux Ratios	
φ_{Po} or TR_0/ABS	$= 1-(F_{50\mu s}/F_M)$ or F_V/F_M
φ_{E0} or ET_0/ABS	$= 1-(F_{50\mu s}/F_M).\psi_0$
ψ_0 or ET_0/TR_0	$= 1-V_J$
Specific Fluxes or Specific Activities	
ABS/RC	$= M_0.(1/V_J) .(1/\varphi_{Po})$
TR_0/RC	$= M_0.(1/V_J)$
ET_0/RC	$= M_0.(1/V_J).\Psi_0$
Phenomenological Fluxes or Phenomenological Activities	
ABS/CS_0	$= F_0$* or other useful expression
TR_0/CS_0	$= \varphi_{Po}(ABS/CS_0)$
ET_0/CS_0	$= \varphi_{Po}.\Psi_0.(ABS/CS_0)$
RC/CS_0	$= \varphi_{Po}.(V_J/M_0).F_0$*
(when expressed per CS_M, F_0 is replaced by F_M; subscript M indicates that maximal fluorescence was used as measure of absorption per cross section)	

The effective quantum yield, relative electron transport rate and the quantum yield limitation in light-adapted photosynthetic samples were calculated using procedures adopted in pulse amplitude modulation fluorometry according to Schreiber *et al.*, (1994) and Smith (personal communication)

Relative electron transport rate

$$\text{PFD x } \frac{\Delta F}{F_M^L} = PFDx\frac{F_M^L - F_S}{F_M^L}$$ (actinic PFD in this experiment was 80 µmol m^{-2} s^{-1})

Where

PFD= photosynthetically active radiation.

ΔF = difference between maximal and minimal fluorescence for light-adapted states

F_M^L = maximal fluorescence for light-adapted states

F_S = steady state fluorescence yield

The relative electron transport rate is computed with the assumption that *P. angolensis* does not constitute an exception from the rule that the optimum quantum yield $\left(\frac{F_V}{F_M}\right)$ is close to a mean value of 0.83 among unstressed leaves (Schreiber *et al.*, 1994).

The calculation of q_P is based on the premise that F_0^L is equal to fluorescence yield of the leaf after 100 s light-adaptation. Hence F_0^L is equivalent to F_S (sometimes termed F) in pulse-modulated fluorescence procedures. q_P can therefore be calculated as

$$q_P = \frac{F_M^L - F_S}{F_m^L - F_0^{dark}}$$

Similarly q_N

$$q_N = \frac{F_M^{dark} - F_M^L}{F_M^{dark} - F_0^{dark}}$$

Quantum yield limitation (see Schreiber *et al.*, 1994)

$$L_{(PFD)} = 1 - \left(\frac{F_M^L - F_S}{F_M^L * \Phi_{Po}^{dark}}\right) = 1 - \left(\frac{F_M^L - F_S}{F_M^L * \left(1 - \frac{F_0}{F_M^{dark}}\right)}\right)$$

Where Φ_{Po}^{dark} is the maximum quantum yield of primary photochemistry in dark-adapted photosynthetic samples.

In the results and discussion that follow, each expression superscripted as indicated below describes the accompanying physiological state i.e.

$\Phi_{Eo}^{dark} = \Phi_{Eo}$ of the dark-adapted state

$\Phi_{Eo}^{L} = \Phi_{Eo}$ of the light-adapted state

$\Phi_{Eo}^{dif} = \Phi_{Eo}$ of the difference between dark- and light-adapted states.

3.3 Results

Chl. a fluorescence transients from P. angolensis seedlings subjected to five water treatments

The typical Chl. *a* fluorescence transients with characteristic J-I-P regions were obtained for each water treatment and are shown in Figure 3.1. Fluorescence kinetics data at 50 µs was taken as: F_0 (when Q_A is fully oxidised) when RC are open; maximal measured fluorescence intensity (F_M) since the excitation intensity (3000 µmol m^{-2} s^{-1}) was sufficient to result in the closure of all RC of PSII (the complete reduction of the primary quinone acceptor, Q_A); the fluorescence intensity at $F_{300\mu s}$ needed to calculate the initial slope, $(dV/dt)_0$ and the fluorescence intensity at 2ms (J-step) and the *I-step* at 30 ms (Figure 3.2).

The Chl. *a* fluorescence transients, in dark-adapted *P. angolensis* leaves, for the 30%, 40%, 60%, 80% and 100% show similar transient shapes although transients from two replicates of the 30% water treatment lie below the transients of the other water treatments after $F_{3(300\mu s)}$ (Figure 3.2).

Chl. *a* fluorescence transients for light-adapted *P. angolensis* leaves have a shape in which the distinctive J-I-P steps are absent (Fig. 3.2) compared to transients from dark-adapted samples.

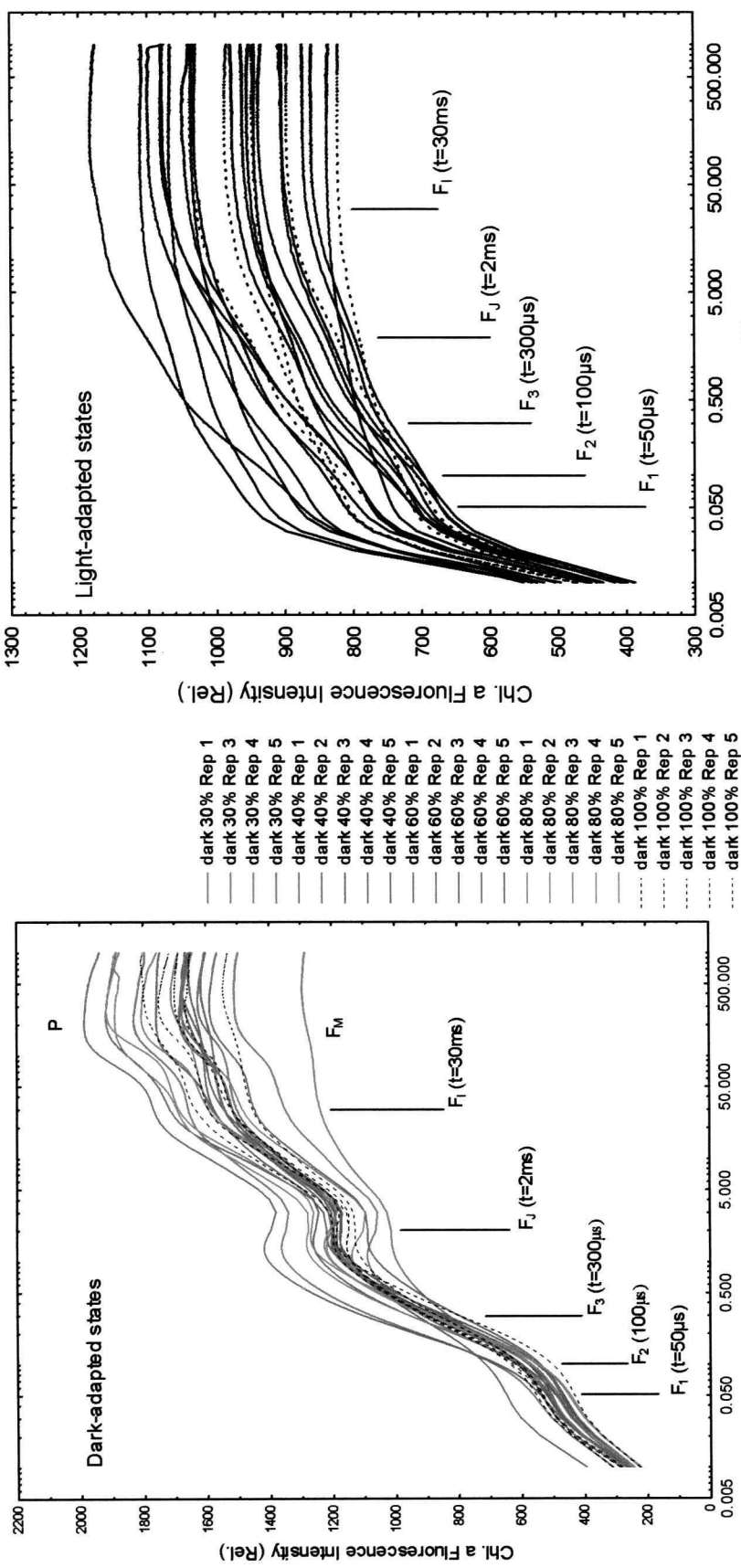

Figure 3.2. Chl. a fluorescence transients exhibited upon the excitation of both dark- and light-adapted leaves of P. angolensis seedlings subjected to 30%, 40%, 60%, 80% and 100% of WHC of the growth medium. Leaves previously excited with a 100% actinic light (3000 µmol m^{-2} s^{-1}) were light-adapted for 100 s with a 80 µmol m^{-2} s^{-1} excitation followed by a 100% saturation intensity. Fluorescence transients are plotted on a logarithmic time scale from 0.01 ms to 1 s. Transients are plotted from the means of days 9 to 11 for each replicate and water treatment.

It is apparent from Figure 3.1 that the shapes of Chl. *a* fluorescence transients of the light-adapted states are different from those of dark-adapted states. Chl. *a* fluorescence transients for the light-adapted photosynthetic samples have an almost flat shape when compared to transients from dark-adapted samples. It is also apparent in Figure 3.1 in which the experimental procedure and distinct differences between the transients from dark- and light-adapted photosynthetic samples are outlined. Due to the fact that no dark-adaptation followed immediately after the first saturating light, the characteristic J-I-P steps in the transient from light-adapted photosynthetic samples are conspicuously absent.

Water treatment effects on Chl. a fluorescence expressions

Results of the analysis of variance and the means of Chl. *a* fluorescence expressions, specific (per reaction centre, RC) and phenomenological (per excited cross section, CS) energy fluxes, flux ratios or yields and de-excitation rate constants are presented in appendix Tables 3.1 to 3.3. Water treatment effects did not significantly affect extracted and technical Chl. *a* fluorescence parameters, specific fluxes or activities, quantum efficiencies or flux ratios and phenomenological fluxes or activities in dark-adapted, light-adapted and the difference between dark- and light-adapted photosynthetic samples for *P. angolensis* nursery seedlings (appendix Table 3.1 to 3.3). Secondly, significant water treatment effects were also not obtained in effective quantum yield, relative electron transport and quantum yield limitation, de-excitation rate constants, structure-function and performance indexes nor for driving forces.

3.4 Discussion

The absence of water treatment differences in all variables reflects the fact that no significant changes occur in the function of PSII for either absorption nor electron flux. Since no water treatment effects occur in the probability that an absorbed photon will move an electron into the electron transport chain $\left(\frac{ET_0}{ABS} \equiv \Phi_{Eo}\right)$, which is directly related to specific energy fluxes i.e., ABS/RC, TR_0/RC and ET_0/RC (Strasser *et al.*, 1999), no statistically significant changes occur in the function of the PS II reaction centers.

Therefore functions related to changes in TR_0/RC^{dark}, which expresses the rate by which an exciton is trapped by the RC resulting in the reduction of Q_A to Q_A^-, remain optimal under these experimental conditions. The fact that there are no differences amongst water treatments in relation to ET_0/RC indicates the probable fact that no changes in Φ_{Eo}^{dark} occur as a result of changes in $(dV/dt)_0$ and TR_0/RC. The fact that water treatment effects on quantum yield limitation and relative rate of electron transport are not significant indicate the fact that the light harvesting complex and the electron transport system synchronise their functions relative to light and water stress. Photodamage to PSII reaction centres, probably due to the concurrent 3% light-adaptation following 100% saturating intensity without dark-adaptation, did not occur as inferred from the lack of differences between dark-adapted and light-adapted Chl. a fluorescence expressions.

Under these experimental conditions, *P. angolensis* seedlings are able to optimise electron activity regardless of the level of water applied (Strasser, personal communication) which is confirmed by the lack of significant differences amongst water treatments for Chl. *a* fluorescence expressions in dark-adapted, light-adapted and the difference between dark-adapted and light-adapted states. Additionally, the species response to water treatments may be due to its persistence in habitats that vary widely in climatic patterns and soil types.

3.5 Conclusion

The assessment of the effect of water treatments on the ability of *P. angolensis* to adapt from dark to light conditions in order to ascertain the likely effects of water treatments on the occurrence of shoot die-back shows that light conditions and water treatments impact on fluorescence yield through changes in the profile of transients. Differences in the shapes of Chl. *a* fluorescence transients of dark- and light-adapted samples are apparent by the absence of the J-I-P steps in light-adapted states. But the difference between dark and light-adapted states do not translate into statistically variable extracted and calculated Chl. a fluorescence variables.

Since saturating light was used (1242 μmol m^{-2} s^{-1}), nonphotochemical quenching and inactivation of RCs may have played a role in determining the patterns of fluorescence induction kinetics as evidenced by the differences in the O-J-I-P steps. The increase in initial fluorescence intensity (F_0 to F_S) after light-adaptation may have been due to a high fraction of the reduced electron acceptor Q_A. F_0 is known to be affected by environmental stresses that cause changes to PSII pigments (Krause and Weis, 1984). Photoinhibition has been associated with a slight increase in F_0 in broken chloroplasts whereas thermal damage of PS II is characterised by drastic increases in initial fluorescence intensity.

The non-significant low height of P in 30% and 100% water treatments reflect the magnitude of the transient block of PSI activity, that causes the rise to P due to a lack of efficient electron acceptors of PSI. Lack of efficient electron acceptors might be due to an increase in the number of inactivated RCs or the transformation of RCs to quenchers through some form of photodamage. The decrease in P may be a result of either enzyme activation or increased ATP/ADP ratio through the stimulation of Calvin cycle activity (Riznichenko *et al.,* 1996).

Decreased electron backpressure in the electron transport chain results in decreased fluorescence yield and increased electron backpressure in the electron transport chain is due to the absence of activated Calvin cycle processes. This results in increased fluorescence intensity. Changes in fluorescence yield resulting from the efficiency of electron transport are largely influenced by variations occurring at the rate constant level of each photochemical and nonphotochemical process.

The lack of significant changes in antennae size (ABS/RC) and electron transport (ET_0/RC) with corresponding non-significant variations in Φ_{Eo}^{dark} may be due to the fact that the remaining functional RCs efficiently maximise the utilisation of trapping flux for electron transport. During senescence of field-grown wheat (*Triticum aestivum* L) flag leaves, Lu *et al.* (2002) found that PSII remained functional, the only change being its down-regulation under steady state photosynthesis.

Down-regulation of PSII was associated with the closure of PSII reaction centres and the occurrence of enhanced xanthophylls cycle-related thermal dissipation in PSII antennae. Therefore, continued closure of RCs greatly influences the structural and functional ability of PSII to effectively utilise excitation energy.

The results indicate the fact that in *P. angolensis*, under these experimental conditions, there is no variable that is sensitive to water treatment used when combined with two light-adaptation regimes. It has been shown from this study that a combination of water treatments and saturating light effects, without an intervening dark-adaptation phase between dark and light, is not sufficient to assess changes in PS II function of *P. angolensis* seedlings. Secondly even though the JIP test makes available many variables that can be used for analysing stress effects, some may not provide sufficient indications of physiological changes within the PSII reaction centres of *P. angolensis*. Therefore, the assessment of water treatment effects on PSII function in *P. angolensis* seedlings should be applied over a longer period exceeding 11 days that was used in this experiment. Additional verification of this inference using empirical data is required as this is the first time that the JIP test is applied in assessing water treatment effects on fluorescence kinetics in *P. angolensis*.

It may be reasonably assumed that the chlorosis observed in the leaves of the species may have a significant impact on the ability of the photosystems to efficiently utilise excitation energy in electron transport. Therefore, *P. angolensis* may have a highly evolved adjustment mechanism that maximises quantum yield and electron flux in situations of saturating light or probable photodamage to the light harvesting system. These processes have evolved to be highly co-ordinated and controlled such that a sudden loss of photosystem functionality is highly unlikely, that is why the gradual progression of physiological processes leading to shoot die-back closely resemble the gradual changes in the environmental factors they are associated with.

The results of this experiment are not sufficiently conclusive to draw conclusions concerning the response of the species to water treatments and the likely role of water availability in shoot die-back

CHAPTER 4

4. GENETIC VARIATION IN SHOOT DIE-BACK AND OTHER TRAITS OF SIXTEEN HALF-SIB FAMILIES OF *Pterocarpus angolensis* DC

4.1 Introduction

Shoot die-back, or shoot senescence, takes place in *Pterocarpus angolensis* saplings annually during the cool, dry season of the tropics and sub-tropical regions in eastern and south-central Africa. The period of low precipitation and low temperature corresponds to the period of dormancy in *P. angolensis* in the Zambesian woodland area. Therefore, the main phenological traits of plants in the Zambesian plateaux (May-July = cold, dry season; August-September = hot, dry season; October-November = early rainy season; December-February = rainy season; March-April = late rainy season) are influenced by rainfall and temperature (Menaut *et al.*, 1995) which is characterized by shoot die-back in naturally regenerated *P. angolensis* seedlings. It has been suggested by Boaler (1966) and Vermeulen (1990) that all the *P. angolensis* seedlings undergo shoot die-back for up to 10 years before they emerge from the suffrutex phase. Variations in phenological traits of *P. angolensis* amongst geographical regions or 'provenances' have not been reported (Vermeulen, 1990).

Ecotypes were observed in Malawian populations of *P. angolensis,* but due to overlapping the differentiation was not clear (Munthali, 1999). The high phenotypic and genetic correlations that were reported to exist between shoot and root traits can be used for initial selection as indicators of the expected seedling growth and survival. The genetic variation that was observed in shoot and root traits may be indicative of the existence of genetic variation in shoot die-back. A positive correlation between shoot die-back and the sizes of the root and shoot has been reported by Munthali (1999) even though the existence of any genetic or phenotypic variation in this trait has not yet been reported. These findings support the view that *P. angolensis* nursery seedlings should produce a sufficiently large and well developed tap-root to minimise the length of shoot die-back.

This experiment was carried out to ascertain the existence of family variation in shoot die-back and other traits of sixteen families of *P. angolensis* from Malawi, Namibia and Zambia and to determine whether such family variation can be used to aid selection amongst families. Family variation was not expected to occur in shoot die-back and other traits of the species.

4.2 Materials and Methods

Seed from sixteen (16) families from Malawi, Namibia and Zambia (Appendix 4.1) was sown and germinated in 1.2 *l* black plastic bags that had a depth of 30 cm, in December 2000 in the Department of Forest Science nursery (33° 56' S, 18° 52' E) of the University of Stellenbosch, South Africa. The daily temperature and humidity in the glasshouse fluctuated greatly depending on the prevailing climatic conditions. The maximum and minimum mean monthly temperatures recorded were 38° C and 6° C respectively over the test period that also covered the winter. Environmental control in the glasshouse was limited to cooling by two thermostatically controlled exhaust fans that were set at 35° C and was supplemented, in summer, by evaporative cooling. The roofing for the glasshouse is made of fibre carbon glass that reduced solar radiation by 30%. Seasonal and diurnal ranges of solar radiation varied based on the seasonal and diurnal patterns obtained in the Western Cape.

Seedlings were raised in sandy-loam soil made up of 2 parts sandy soil mixed with one part of fine compost, with a pH (KCl) of 6.2 (cf. appendix Table 2.1.1). The potting mixture was inoculated with soil containing mycorrhiza from a *P. angolensis* site near Nelspruit. Seed pre-treatment involved nicking of the seed by removing a part of the seed coat (Kasumu, 1998; Munthali, 1999).

The experimental design was as follows
Replications = 6
Number of seed sources = 3 (Malawi, Namibia and Zambia)
Number of half-sib families = 16 (Malawi=9 families from 4 accessions, Namibia=4 families from 1 accession, Zambia=3 families from 1 accession)
The sixteen half-sib families that were tested are listed in Table 4.1.

Water was applied in the morning and cool hours of the late afternoon at about 70 ml per plant during the hot summer season. The water regime was scaled down to 35 ml per plant every 48 hours during the winter season. The scaled down water regime was necessitated by the dormancy phase of the species and the reduced evapo-transpiration rates that occur during winter.

Seedlings were supplemented with the application of 10 ml of a 2 g/*l* solution of ammonium sulphate containing 21% N [210 g/kg N (w/w)] per seedling on a weekly basis in the first season of growth. Thereafter the application was reduced to one application per month. Iron chelates (with 6% Fe) were mixed with water at 400 mg/*l* and applied at 1 *l*/10 m^2 of glasshouse space. The iron foliar spray was applied very lightly as chemical scorching occurred when the solution left a coating of iron on the leaf after drying. Improvement after iron application was observed within two weeks. Additionally seedlings were raised on a commercial fertilizer, Multifeed® which was applied as a foliar application. In all foliar applications a wetting agent was mixed with the nutrient solution at 1 ml/5*l*.

Non-nutritional glasshouse problems with the seedlings occurred with respect to aphids, mites and red spiders which were dealt with by the application of Metasystox R® at 2 ml/*l* for aphids and mites, and cyhexatin 600 SC® at 1 ml/2*l* for red spiders.

Seedlings were assessed over two shoot die-back seasons in October 2001 and September 2002. Late spring assessments were done to classify only those seedlings as displaying die-back that had no leaves in winter but that sprouted again after winter. The total number of seedlings used in the 2002 assessment is the surviving portion from 2001 and does not include the mortality percentage. Thereafter all surviving seedlings were assessed for stem height, root collar diameter, taproot length, and shoot and root dry weight (termed biomass in the discussion) in February of 2003. Seedlings were separated into stem and root, and cleaned and treated as described by Kasumu (1998) and oven dried at 80°C for 48 hours.

Table 4.1. Provenances and families used in the determination of family variation in shoot die-back

COUNTRY	PROVENANCE	FAMILY[a]	LATITUDE	LONGITUDE	ALTITUDE (in m a.s.l)	RAINFALL (mean annual in mm)	TEMPERATURE (mean annual in °C)	VEGETATION type
Malawi	Skull Rock (Mangochi)	M29 M32	14° 25' S	35° 28' E	1014	-	Min. 16-18 Max. 28-30	Grassland & open woodland (*Brachystegia*)
	Mbongondo (Rumphi)	M19 M82 M87	11° 03' S	33° 52' E	1210	800-1200	Min. 14-16 Max. 28	Grassland (*Schrebera trichoclada*)
	Ngara (Karonga)	M70 M74	10° 15' S	34° 06' E	590	800-1200	Min. 20 Max. 28-30	Woodland (*Pterocarpus angolensis*)
	Phalombe (Mulanje)	M119 M125	15° 50' S	35° 40' E	970	800-1200	Min. 14-16 Max. 24-26	Woodland (*Eucalyptus*)
Namibia[b]	Kanovlei (Namibia)	N5 N7 N8 N10	19° 10' S	19° 23' E	1180	400-600	Min. 15-26* Max. 25-33*	
Zambia[b]	Kalulushi district (Zambia)	Z5 Z16 Z19	12° 50' S	28° 03' E	1250	1200	Min. 7-18 Max. 25-30	Miombo (*Brachystegia, Julbernadia, Isoberlinia*)

[a]/: M=Malawi (originally MKCN); N=Namibia, Z=Zambia

[b]/: Source of geographical and climatic data: Jackson (1961)

Root collar diameter was measured using an electronic diameter calliper. Shoot height and taproot length were measured with a 30 cm ruler prior to oven drying whereas dry weight was determined after oven drying. Each seedling was examined to determine whether it died back or not. The count was later determined as a proportion of the total number of seedlings per plot. Counts of shoots that died back, in per cent, were then transformed using arcsine transformation $y = \arcsin\sqrt{x}$ or $y = \sin^{-1}\sqrt{x}$ (Ott, 1993) where y is the transformed variable and x is the variable to be transformed. The reciprocal transformation, $y = \frac{1}{x}$ (Ott, 1993), was used for shoot height, root collar diameter, taproot length, shoot and root dry weight, and the ratio of shoot to root dry weight.

The mixed effects model for the analysis was $Y_{ijk} = \mu + R_i + P_j + (RP)_{ij} + F_{k(j)} + \varepsilon_{ijk}$, where

Y_{ijk} = is the response obtained at the i^{th} replicate, j^{th} provenance and k^{th} family,

μ = is the mean of the population

R_i = the i^{th} level of the random effect of replication (i=6)

P_j = the j^{th} level of the fixed effect of provenance (j=6)

$(RP)_{ij}$ = the interaction term between replication and provenance

$F_{k(j)}$ = the k^{th} random family effect (k=16) nested within the j^{th} level of the fixed effect of provenance and the ε_{ijk} are assumed to be normally distributed with mean 0 and unknown variance, and independent. The form of the variance component analysis for shoot die-back tests of half-sib families is presented in Table 4.2. The provenance effect was accounted for prior to estimating the family variance component in plant parameters (Williams *et al.*, 2002).

Table 4.2. Form of the analysis for estimation of variance components

*SOV	DF	MS	F	Expected Mean Square
Replication (R)	(r-1)	MS_R	MS_R/MS_w	$\sigma^2_w + npf\sigma^2_R$
Provenance (P)	(p-1)	MS_P	MS_P/MS_{RP}	$\sigma^2_w + nf\sigma^2_{RP} + nrf\sigma^2_P$
R*P	(r-1)(p-1)	MS_{RP}	MS_{RP}/MS_w	$\sigma^2_w + nf\sigma^2_{RP}$
Family(Provenance)	p(f-1)	$MS_{F(P)}$	$MS_{F(P)}/MS_w$	$\sigma^2_w + nr\sigma^2_{F(P)}$
Error	(k-1)	MS_w		σ^2_w

*SV, DF, MS = source of variation, degrees of freedom and mean square

** σ^2_w = error variance component

** $\sigma^2_{F(P)}$ = half-sib family variance component

** σ^2_R = variance component of the replication

** σ^2_P = provenance variance component

** σ^2_{RP} = replication and provenance interaction variance component

** n = number of trees per plot.

The degrees of freedom described in Table 4.2 are not as presented in the results of the analysis of variance due to the fact that families are not balanced within provenances. The variance component estimate for half-sib families was computed by

$$\frac{MS_{F(P)} - MS_W}{nr}$$

where

$MS_{F(P)}$ = mean square of the family effect nested within the provenance

MS_w = residual mean square.

The standard error of family variance component was calculated according to Anderson and Bancroft (1952)

$$S.E(\sigma^2_{F(P)}) = \sqrt{\frac{2}{(nb)^2}\sum\frac{MS_i^2}{df_i + 2}}$$

Where

$S.E(\sigma^2_{F(P)})$ = standard error of family variance component

nb = coefficient of the family variance component

MS_i = mean squares of the sources of variation involved in the determination of $\sigma^2_{F(P)}$

df_i = degrees of freedom of mean squares involved in the determination of $\sigma^2_{F(P)}$.

The family variance of the half-sib (open pollinated) families estimates ¼ of the additive genetic variance only if each seedling has the same maternal parent and unrelated pollen parent (Williams *et al.*, 2002). Since this rarely occurs in nature, the coefficient of relationship (r) of each *P. angolensis* seedling with another seedling is taken as ⅓ due to the fact that there are likely to be seedlings in the family with a common father or with related pollen parents. Additionally, there are some seedlings that arose out of selfing, therefore making the coefficient of relationship larger than ¼ (Squillace, 1974; Williams *et al.*, 2002). *P. angolensis* flowers were taken to be hermaphrodites – having both stamens and pistils on the same flower – as determined from Boaler (1966) and Vermeulen (1990) even though no mention is made of the type of flower nor whether cross-pollination, selfing or both occur. In open-pollinated situations (such as *P. angolensis*), Squillace (1974) identified four kinds of likely relatives: self full-sibs, full-sibs, half-sibs and a self and an outcross which are termed "self half-sibs". Therefore, the type of relatives to each seedling in *P. angolensis* is difficult to classify due to lack of research results. It is assumed, for the purpose of this study, that chances of self full-sibs being present in our sample cannot be discounted.

Therefore, narrow sense heritability (h^2) estimates for shoot die-back traits in half-sib families were calculated for each year of assessment as follows

$$h^2 = \frac{\sigma^2_F / r}{\sigma^2_{RP} + \sigma^2_F + \sigma^2_w}$$

where

h^2 = is the narrow sense heritability of shoot die-back traits in half-sib families

σ^2_F = family variance component

r = coefficient of relationship (r=⅓).

Negative variance component estimates were treated as if they were zero.

Statistical calculations for significance tests and mean comparisons were carried out in SAS Enterprise Guide Release 1.3 (SAS Institute, 2001). The reliability of the heritability estimate was assessed through calculation of its standard error by using the Dickerson approximation that assumes that the denominator of $h^2 = \frac{\sigma_F^2 / r}{\sigma_T^2}$ is a constant (Williams *et al.*, 2002). Therefore

$$S.e.(h^2) \approx \frac{S.e.(\sigma_F^2) / r}{\sigma_T^2}$$

where

S.e.(h^2) = standard error for estimated heritability

S.e.$\left(\sigma_F^2\right)$ = standard error of the family variance

σ_T^2 = estimated proportion of total phenotypic variance.

4.3 Results and Discussion

The results of the experiment are summarised in the analysis of variance and variance component estimates for shoot die-back (appendix Table 4.1, 4.2 and 4.3) and comparison of means (appendix Table 4.2 and 4.4). Graphical presentations of mean responses are in Figure 4.1 and 4.2. The occurrence of shoot die-back in the first shoot die-back season, 2001, was not significantly different amongst the 16 half-sib families. This changed during the second shoot die-back season in 2002 in which half-sib families showed significant differences (appendix Table 4.1). Significant half-sib family variations were also observed in shoot height, root collar diameter, shoot and root biomass, and the ratio between shoot and root biomass (appendix Table 4.3).

Narrow sense heritability was estimated at 0.42 for shoot die-back in 2002 (appendix Table 4.1) and more than 0.30 for shoot height, root collar diameter and shoot and root biomass (appendix Table 4.4).

The occurrence and genetic basis of shoot die-back in half-sib families of P. angolensis

The Malawian half-sib family, M87, had significantly higher shoot die-back means than M125, M29, M32, N10, N7 and N8 in 2002 (Fig. 4.1.1 and appendix Table 4.2). Even though 50% of seedlings showed shoot die-back in 2001 for M87, an increase in the proportion of seedlings showing shoot die-back in M87 was observed in 2002 in which 91% died back.

The main phenological traits of plants in the Zambesian plateaux, and in other tropical and subtropical regions with alternating dry and rainy seasons, are influenced by rainfall and temperature (Stern and Roche, 1974; Menaut *et al.*, 1995). Over several generations, plants adapt to the alternating dry and wet seasons and their responses, compared to other ecotypes, may be significantly different. It has been observed by Stahle *et al.* (1999) that the phenology of *P. angolensis* is strongly synchronised with the seasonality of rainfall, even though the amount and length of the rainy season are not specified. The Malawian (M87) half-sib family lies further north in relation to the Namibian half-sib families and has comparatively higher mean annual rainfall and temperature than the Namibian half-sib families. Climatic variables are important considerations when an inter-provenance or seed source comparison is made. These variables are not considered in isolation of soil factors. Strong correlations of the components of the soil complex with climatic factors exist since soil genesis with its corresponding influence on individual soil factors is significantly affected by seasonal variations in precipitation and temperature (Stern and Roche, 1974).

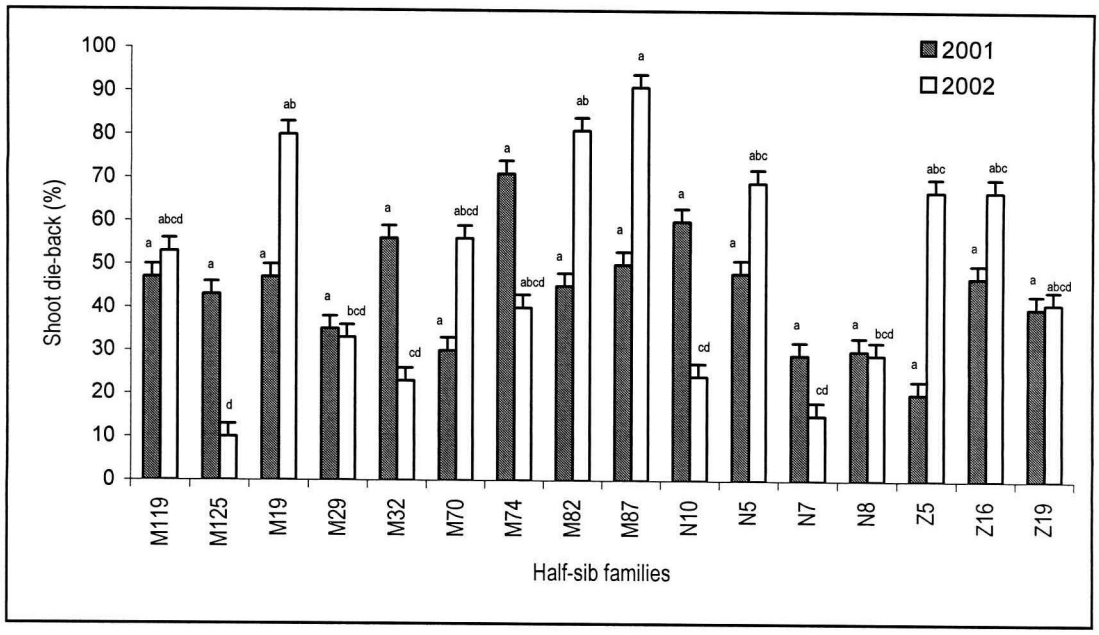

Figure 4.1. Mean shoot die-back half-sib families of P. angolensis. Vertical bars are standard errors of unadjusted population means; bars with the same letter indicate a lack of a significant difference between the shoot die-back means.

Phenotypic manifestations of shoot die-back may have a variable genetic basis in a species that occurs over such a wide and diverse range. The 0.42 narrow sense heritability, h^2, obtained in 2002 for shoot die-back in half-sib families of *P. angolensis* (appendix Table 4.1) indicates the proportion of the total phenotypic variation in shoot die-back that is attributable to genetic (additive genetic variation) as opposed to other effects.

Secondly, shoot die-back, therefore, is a quantitative trait that shows additive genetic variation in the population and will be passed on from parent to offspring. Polygenic or complex traits are affected by multiple genetic and environmental factors in their inheritance (Falconer and Mackay, 1996; Lynch and Walsh, 1998; Cooper *et al.*, 2002) and this may be why the response of *P. angolensis* seedlings to declining temperature elicits different responses, in all seed sources, such as complete or partial shoot die-back. This therefore indicates that shoot die-back is not a random physiological event.

The expression of shoot die-back in this species has a discernible genetic phenomenon attributable to the resemblance between relatives. However, the degree of resemblance in shoot die-back varies with the onset and severity of the dry season as well as the cool season. Even though parent trees do not undergo stem senescence, the synchronised occurrence of leaf fall (in mature trees) and shoot die-back (in saplings) is a major resemblance between offspring and parents since siblings also show leaf fall prior to stem senescence. Probably a closer examination of parental shoot die-back history may reveal a similar pattern in shoot die-back in siblings.

The gradient in expression of shoot senescence in *P. angolensis* indicates the involvement of senescence down-regulated genes (SDGs) and senescence-associated genes (SAGs). Genes whose expression is decreased during the process of senescence (SDGs) include those that encode proteins involved in photosynthesis (Taiz and Zeiger, 1998). Other genes whose expression is induced during senescence (SAGs) include genes that encode hydrolytic enzymes such as proteases, ribonucleases, and lipases. The manifestation of changes in the expression of the effect of SDGs and SAGs is slow and long-drawn, probably occurring from the time of leaf fall to the completion of shoot senescence. In order to improve the phenotype of the trait such as shoot die-back in *P. angolensis*, a combined analysis and understanding of the genetic architecture and heritability of the trait of the reference population would be necessary.

Such a costly exercise may not be justified by economic returns from a genetically improved *P. angolensis* breeding programme. Phenological observations in three different geographical regions of the species indicate that leaf flush in mature plants, which follows the period of dormancy, occurred relatively earlier in northern than in southern habitats (Boaler, 1966; Vermeulen, 1990). The topographical location was also observed to influence the leafless period, which was relatively shorter at low altitudes and high rainfall areas.

The reported existence of ecotypes in the Malawian population of *P. angolensis*, without differentiation due to overlapping (Munthali, 1999), may not be an isolated feature of the species even though the near-absence of a taxonomic subdivision indicates that, botanically, there is very little variation in the species (Von Breitenbach, 1973). Differentiation into ecotypes more likely occurs in common and widely distributed species (Stebbins, 1967) such as *P. angolensis* than in rare, local or endemic species. The overlapping observed by Munthali (1999) that obscures genecotypic differentiation may probably be due to relatedness amongst ecotypes. Assuming relatedness exists, it may be difficult to distinguish ecotypes by physiology even though morphological differentiation may exist between or amongst distantly spaced assemblages.

This may be the reason why variability between provenances and half-sib families for morphological characteristics were obtained by Musokonyi (1998), Munthali (1999) and in this study. Significant half-sib family variations in root traits are partly reflective of soil influences in different habitats apart from genetic influence. Variations in root characteristics may exist among genotypes of an assemblage, and these differences significantly influence a population's competitive ability and influence (Stern and Roche, 1974). Shoot die-back is principally physiological followed by non-reversible morphological changes. It shows lower intra-specific variation even though it seemingly exhibits regularities across regions and may or may not be connected to segregated adaptation of different populations to specific ecological conditions.

Whether ecotypes exists over the range of *P. angolensis* is not yet known, the difficulty being the recognition and identification of well-marked assemblages of genetic variants due probably to a more or less existence of morphologically and ecologically intermediate populations of related individuals or groups. A typical example of the similarity in certain physiological traits is the occurrence of progressive die-back and the formation of epicormic shoots leading to death in mature trees. The decline of mature trees that show similar patterns and progression was observed in Botswana, Zambia and Zimbabwe (FAO, 1994). It is a vascular wilt disease of *P. angolensis* caused by the soil borne fungus (*Fusarium oxysporum).*

The fact that this particular attribute occurs across regions of diverse ecological conditions indicates common response patterns to the fungus. Additional validity of morphological diversity relative to topographical and climatic factors is provided by the results of Musokonyi (1998) and Munthali (1999) whose work with seedlings showed a positive correlation between height growth and longitude, latitude and rainfall amount. Genetic variation patterns over the *P. angolensis* range, or within a sub-population of it, are not known except for the Malawian half-sib families that are reported by Munthali (1999). In such a wide distribution, genetic variation is expected to be higher in a large geographic range and especially when the distribution is discontinuous (Barnes, 1990).

Genetic variation is more likely to be higher along the periphery of the species distribution and unexpected but important alleles may occur in even the smallest isolated populations (Stebbins, 1967; Barnes, 1990). Isozyme studies on the genetic structure of tropical woody species have shown the existence of high levels of genetic variation and intra-population differentiation (Liengsiri *et al.,* 1995). Similar studies in the genus *Pterocarpus*, in *Pterocarpus macrocarpus* Kurz., found high correlation coefficients between genetic and geographic distance. This suggested that isolation by distance contributes to population differentiation in the species or the genus (Liengsiri *et al.,* 1995). The fact that only M87 from Malawi was significantly different from other some of the half-sib families may be indicative of a "deviance". This may be indicative of the lack of or existence of a weak family variation, meaning that it is a common occurrence over the species distribution. Secondly, the evaluation of shoot die-back at glasshouse level may confound finely tuned evolutionary and adaptive responses that are manifested differently when critical environmental variables such as moisture are varied.

The absence of significant differences between half-sib families from the Malawi and Zambian sites may be indicative of individual tree responses in terms of shoot die-back, which may be in support of the emphasis by Barnes (1990) that in assessing provenance performance, individual trees should be randomly rather than phenotypically selected. Random selection of individual families for assessing shoot die-back is important since this trait is only restricted to saplings and not mature trees.

Half-sib family differences in shoot height, taproot length, root collar diameter, shoot and root biomass

Shoot height, root collar diameter, root and shoot biomass, and the ratio between shoot and root biomass showed significant variations amongst the 16 half-sib families (appendix Table 4.3). Differences between means of each trait that were significant are apparent particularly with regard to M29 (appendix Table 4.5).

The lack of significant variations (Figure 4.2) in taproot length may be related to the size of the container in which seedlings were grown. It was observed that taproot length does not increase with time (cf. chap.2, section 2.1) due to lack of taproot extensibility. Therefore, lack of family variation is mainly due to the restriction imposed by the 30 cm deep container.

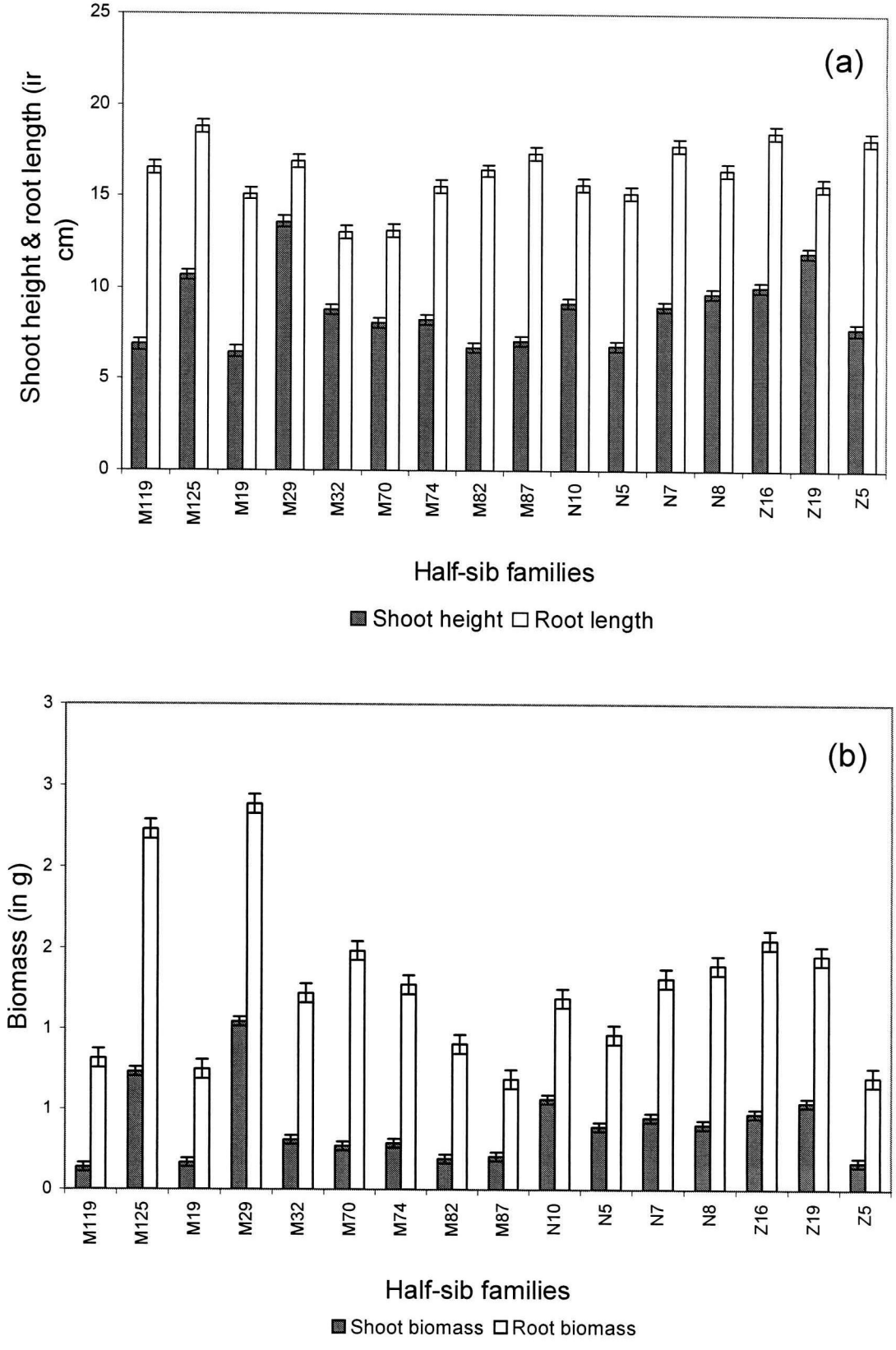

Figure 4.2a. Mean (a) shoot height and taproot length (b), shoot and root biomass for individual half-sib families of P. angolensis. Vertical bars are standard errors of unadjusted population means for each variable.

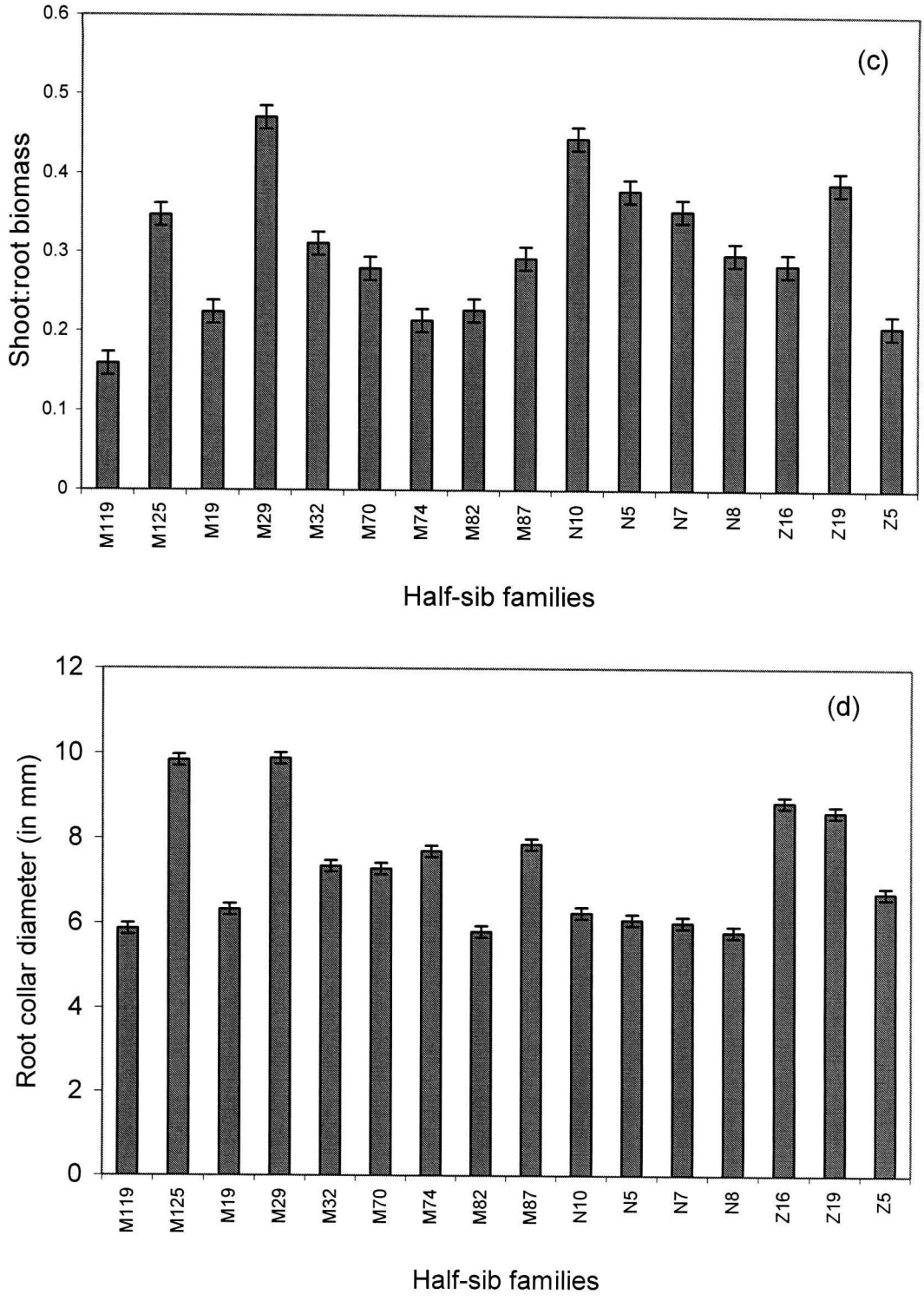

Figure 4.2b. Ratio of shoot to root biomass and root collar diameter for individual half-sib families of P. angolensis. Vertical bars are standard errors of unadjusted population means for each variable.

There was no significant phenotypic correlation between shoot die-back for 2001 and 2002 with shoot height, root collar diameter, shoot and root biomass, and the ratio of shoot to root biomass (Table 4.3).

Table 4.3. Phenotypic correlation of shoot die-back and other plant traits

Year of assessing sdb[1]	SHOOT DIE-BACK AND OTHER PLANT TRAITS							
	2001 Sdb[1]	2002 Sdb[1]	RCD[2]	Shoot Height	Taproot Length	Shoot dw[3]	Root dw[3]	Ratio[4]
2001 (Sdb[1])		0.12	-0.09	0.06	-0.07	0.13	0.08	-0.04
2002 (Sdb[1])	0.12		-0.05	-0.03	-0.09	-0.06	-0.15	0.10

[1]/: Sdb = Shoot die-back; [2]/: RCD = Root collar diameter; [3]/: dw = Dry weight
[4]/: Ratio of shoot dry weight to root dry weight

Heritability of shoot height, root collar diameter, shoot and root biomass, and ratio of shoot to the root biomass of P. angolensis seedlings

High narrow sense heritability estimates that were obtained in this study for shoot height, root collar diameter and root biomass concur with those of Munthali (1999) in which h^2 estimates of more than 0.20 were obtained. The higher root collar diameter and root biomass narrow sense heritability estimates may be due to the restricted container depth which leads to higher variability in root parameters. Large height and diameter growth in mature trees has been reported to be a characteristic of populations from Malawi, Mozambique, Tanzania and Zambia whilst short and small diameter trees are more common in South Africa (Vermeulen, 1990). The characterisation of the population may be indicative of taller trees having large diameters too such as was reported by Munthali (1999) when seedling shoot height was correlated with root collar diameter.

4.4 Conclusion

Phenotypic variation, for traits such as shoot die-back, is likely to be associated with environmental differences in *P. angolensis* that grows over a wide range of climatic and edaphic conditions. Consequently, the significant differences obtained between the half-sib families may partly be associated with differences in topographical and climatic conditions. Basic differences in latitude, longitude and altitude as well as mean annual rainfall may have an effect on the physiological response of plants to seasonal changes in the climate. Half-sib families which originate from a drier geographic region, are more than likely to respond to any minimal change in climatic conditions due to the harshness of the habitat to which they have become adapted.

The Malawian families grow in wetter and cooler habitats and are therefore more than likely to be less sensitive to minor climatic variations. Previous findings of genetic variation, in seedling morphological traits, amongst families from Malawi indicate the possible existence of genetic differences within provenances or the existence of genetically distinct ecotypes or random genetic variants within ecotypes in *P. angolensis* habitats. The possibility of differences based on topographical and climatic considerations, as in the case of shoot die-back, makes it possible to select from half-sib families within provenances that have lower or higher frequencies of shoot die-back. Under glasshouse conditions, this view is feasible but field conditions rarely produce any differentiation in the frequencies of shoot die-back between individual plants and seed sources (cf. Chap. 2, section 2.4). Making selections under field conditions by using shoot die-back as a criterion requires the incorporation of other variables such as the number of plants undergoing shoot die-back at the beginning of and within the cool dry season, how many regenerate shoots and when, as well as the relative growth of the saplings after shoot die-back. The latter aspect is related to the fact that shoot dry weight is positively correlated with root dry weight (cf. Chap.2, sub-section 2.1), meaning that larger shoots are more likely to have larger roots and vice versa. Therefore, combined selection of shoot die-back and other growth characteristics such as post die-back survival and biomass should be used in making phenotypic selections.

The strong genetic variation obtained in shoot die-back is an indication that re-sprouting is under a strong genetic control. This is due to the fact that the assessment of shoot die-back focuses on the ability of seedlings that have died back to re-sprout thus dissociating die-back from mortality and survival. The fact that in seedlings that had died back, roots with high dry weights produce shoots with a high dry weight (cf. Chapter 2, section 2.1) is an indication that shoot die-back has beneficial effects on biomass increment. High phenotypic and genetic correlations between shoot and root traits were also obtained by Munthali (1999). This concurs well with the high heritabilities obtained for root and shoot biomass which places the two traits under genetic control too. This trend indicates that the survival of *P. angolensis* seedlings depends on the fact that it retains the ability to regenerate a new shoot after die-back. Since all seedlings under field conditions undergo shoot die-back, it may be inferred that survival under field conditions is genetically controlled.

Even though the results of this study provide a limited picture of the genetic basis of shoot die-back due to the small size of the sample, the high heritabilities obtained, with acceptable standard errors, warrants further research which will validate some assumptions made in this study.

CHAPTER 5

5. CONCLUSION AND RECOMMENDATIONS

5.1 Summary of aim and objectives of experiments conducted

A study involving the assessment of the effect of factors related to shoot die-back on growth and physiological responses of *P. angolensis* seedlings was undertaken. This was carried out to provide information that would broaden our understanding of the shoot die-back phenomenon and to enhance our ability to regenerate the species. The study was multi-faceted and encompassed the following:

1. Assessment of biomass accumulation; anatomical characteristics of the shoot apical meristem; foliar, stem and root concentrations of micro- and macro-nutrients associated with each phenophase.

2. Effect of seedling age and seed source on the occurrence of shoot die-back under field conditions in seedlings raised in the nursery.

3. Water treatment effects on Chl. *a* fluorescence traits of *P. angolensis* seedlings obtained by assessing the fluorescence yield of photosynthetic samples subjected to dark- and light-adaptation.

4. Genetic variation in shoot die-back and other traits of sixteen half-sib families of *P. angolensis* from Malawi, Namibia and Zambia grown over two die-back seasons.

5.2 Conclusions

5.2.1 Description of shoot die-back

Shoot die-back in *Pterocarpus angolensis* refers to stem die-back following leaf fall. Variations exist in its progression and manifestation under glasshouse and field conditions. In the glasshouse, not all plants show shoot die-back even though shoot die-back takes place in all saplings growing under field conditions. Due to the fact that *P. angolensis* is deciduous, i.e. sheds leaves during the dry and cool season, it is reasonable to refer to this phenomenon as *stem die-back* which appropriately describes the part of the plant that shows die-back. Leafless plants may occur early in the growing season, i.e. during the rainy season, even though they do not immediately show stem die-back.

Complete shoot die-back occurs progressively from the shoot apex downwards. The whole stem down to the root collar senesces. A general and extensive leaf chlorosis starts after leaf maturation towards the cool dry season. Sometimes chlorosis overlaps into the shoot die-back season and thus occurs from late summer into early winter or the whole of winter. Leaf chlorosis is preceded by the shrinkage and browning of terminal and axillary buds. Axillary buds along the main stem shrink and become inconspicuous prior to and during the course of leaf chlorosis and shedding. Thereafter bud location and existence are only noticed through the leaf and terminal bud scale scars, respectively. Shoot elongation, both apical and lateral, generally ceases as does leaf expansion in seedlings in which leaf chlorosis and shedding are about to be initiated. Under glasshouse conditions, shoot die-back is categorised into three broad sub-groups

- *Complete shoot die-back (termed "shoot die-back")* in which the whole shoot from the apical bud to the shoot base senesces. The seedling container in such situations seemingly contains no sign of the presence of a plant and may be mistaken for mortality;

- *Incomplete shoot die-back* affects more than half of the shoot and leaves a short part of the shoot protruding from the soil. The surviving portion of the shoot does not contain lateral branches compared to what is obtained in partial shoot die-back;

- *Partial shoot die-back*: less than half of the top part of the shoot senesces. The remaining live portion will usually survive the cool season but may only play a supportive role in the life of the successive growth season's shoot.

The last two shoot die-back categories are not manifested under field conditions. The fact that all seedlings die-back under field conditions is one major difference in growth characteristics between glasshouse and field conditions. Stem senescence in the field involves the whole stem but a short dead 'stump' may remain, above the root collar but not protruding above the soil surface. Generally no stump remains and the next shoot generation is from adventitious buds located on the top part of the root. In the glasshouse, only in complete shoot die-back does shoot regeneration originate from adventitious buds. The common trend is that shoot regeneration arises from axillary buds located on the remaining part of the previous season's stem.

Therefore, shoot die-back in *P. angolensis* can reasonably be described as *the gradual and seasonally permanent termination of leaf and stem life processes that occurs during the cool and dry tropical seasons.* Under glasshouse conditions, the description may not include the term "dry" but focuses on other environmental variables.

5.2.2 Implication of findings in regenerating *P. angolensis*

- Lack of changes in the size of the shoot apical meristem in glasshouse seedlings is most likely brought about by an almost uniform moisture regime. Irrigating the species may not be beneficial under field conditions since it will confound the naturally occuring phenomenon of shoot die-back.

Since desiccation of plant tissues impacts on their relative size, the size of the shoot apex will invariably change during the dry and cool season under field conditions – which is normal and natural.

- The decline of shoot biomass and the modest increase in root biomass imply that management of seedlings in the nursery should concentrate on developing nursery protocols that will optimise root growth. Seedlings should be raised in sufficiently deep containers, with a depth of 30 cm or more, and not kept in the nursery for more than 2 months. This will produce a seedling that is still young but with a root system that is not highly coiled at the base that may hinder adequate root growth in the field.

- Selecting against shoot die-back might not be the ideal silvicultural management of *P. angolensis* since it is genetic, occurs in all seed sources and planting stocks of different ages as well as in direct seeding. It is advisable to select for root collar diameter because it is a good predictor of root size. Secondly, assessments of root collar diameters are not harmful to the plant since only shallow digging would be undertaken.

- If selecting for shoot die-back is adopted in the nursery, great care must be exercised since not all seedlings die back under nursery conditions. A period of simulated drought is ideal to remove the confounding effect of irrigation in winter. It is advisable, at the present state of scientific knowledge, to allow the natural progression of shoot die-back to occur. But efforts should be concentrated on ensuring that factors that may hinder the successful regeneration of a shoot in the following growing season are removed or adequately addressed.

- It is not ideal to water the seedlings during the period of shoot die-back, or the dry season. Progenies from healthy and fast growing families must instead be selected for field planting. Sickly and stunted, or shorter than normal seedlings, are more than likely to perform poorly under field conditions.

Silvicultural interventions that focus on obtaining a bigger root, in terms of volume and depth, remain the most important determinant of a successful plantation programme due to their likely effects on shortening the shoot die-back period as well as post die-back seedling mortality.

5.3 Recommendations

The results that were obtained revealed gaps in information concerning shoot die-back. The availability of this information will aid in understanding the shoot die-back phenomenon and enhancing the silvicultural management of *P. angolensis* at nursery and field levels. Therefore, the following recommendations are made

- Analysis of the storage capacities/qualities of the carrot-like tuber and the thinner part of the tap-root as well as laterals which will assist in understanding the probable advantages of containers that promote root extensibility with a poor carrot-like formation or which promote a higher tuber formation with less root extensibility.

- *In situ* phenophasic assessment of Chl. *a* fluorescence, mineral nutrient cycles and biomass allocation in the foliage of saplings (and suffrutices) and mature trees.

- Evaluation of the impact of water (using the JIP test) and fertiliser on shoot die-back and general growth, particularly root biomass.

- Conduct long-term field observation trials for occurrence of shoot die-back and estimate the relative time at which the phenomenon ceases to occur (in relation to root biomass, carbohydrate reserves and changes in height growth) in order to make available research information on annual shoot and root growth characteristics.

- *In vitro* or hydroponic cultures to understand root growth characteristics as well as the influence of synthetic hormones and individual essential nutrients on shoot die-back and biomass allocation.
- Removal of previous season's shoot, IAA and apical dominance, under conditions of incomplete or partial die-back.

- Evaluation of the relationship between shoot die-back and root and shoot growth.

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APPENDIX

Table 2.1.1. Particle analysis and chemical characteristics of the soil that was used as the potting mixture for growing *P. angolensis* in the glasshouse

Depth (cm)	pH (KCl)	Resistance (Ohm)	H (cmol/kg)	Stone (Vol.%)	Soil type	P (mg/kg)	K (mg/kg)
30	6.2	350	0	8	sand	139	

Exchangeable cations							
cmol/kg				mg/kg			
Ca	K	Mg	Na	B	Cu	Mn	Zn
2.06	0.70	0.73	0.45	0.24	0.74	0.70	4.70

Table 2.1.2. Analysis of variance for testing differences in seed source and seedling age effects on shoot and root biomass, shoot height, root lengths and ratios of biomass yields as well as shoot height and root length of glasshouse grown seedlings of *P. angolensis*

SOV[a]	DF[a]	MEAN SQUARE							
		Shoot height (1)	Root length (2)	Ratio[b] [(1)/(2)]	Shoot dry weight (3)	Root dry weight (4)	Ratio [(3)/(4)]	Total dry weight	Root collar diameter
Block	5	3306*	14.22*	0.0001*	0.094*	0.195*	0.247*	0.265*	0.121NS
Seed source	2	8082*	8.89NS	0.0008*	0.342*	0.019NS	1.080*	0.104*	2.175*
Seedling age	3	4665*	39.69*	0.0008*	1.888*	0.420*	14.79*	0.024NS	1.032*
Error	321	671	4.58	0.00003(320)	0.023	0.018	0.061(310)	0.027	0.054

[a]/: SOV= source of variation; DF= degrees of freedom

[b]/: Ratio of shoot height to root length, shoot dry weight to root dry weight

**/: (*)= significant (p=1%) and (NS)= not significant*

(): Number in parenthesis is error DF

Table 2.1.3. Analysis of variance for regression of biomass yield, shoot height, root length, ratio of biomass yields on seedling age as well as shoot height and root dry weight of glasshouse grown seedlings of *P. angolensis*

SOV[a]	DF[a]	MEAN SQUARE										
		y	Shoot height	Root length	Shoot dry weight	Root dry weight	Total dry weight	Ratio[b]	Root length	Shoot dry weight	Root collar diameter	Root dry weight	Root dry weight
		x	Seedling age	Seedling age	Seedling age	Seedling age	Seedling age	Seedling age	Shoot height	Root dry weight	Shoot height	Shoot height	Root collar diameter
Regression	2		6717^{***}	93947^{***}	2.69^{***}	5.54^{***}	0.138^{NS}	51^{***}	290^{NS}	0.470^{***}	48^{***}	4.31^{***}	8.91^{***}
Error	328		755	4012(329)	0.027	0.154(330)	0.249(329)	0.189	4303	0.040	1.673	0.147	0.118
Adj. R^2			0.05	0.064	0.375	0.096	-0.003	0.620	-0.003	0.061	0.148	0.151	0.318
Parameter Estimates	Intercept		98	239	0.807	0.342	0.728	3.099	204.248	0.255	6.015	0.492	0.382
	β_1		-7.063	-5.139	-0.142	0.039	0.040	-0.638	-0.033	-0.146	-0.029	-0.003	-0.086
	B_2		0.365		0.007		-0.002	0.034		0.149	0.0003	0.00006	0.021

[a]/: *SV= source of variation; DF= degrees of freedom;*
[b]/: *Ratio of shoot dry weight to root dry weight*
***/: (***)= very significant (p=0.1%) and (NS)= not significant.*

Table 2.1.4. *Pterocarpus angolensis* seedling root growth in 2.4m long plastic tubes grown for 10 months

Plastic tube dimensions: 240 cm x 8.5 cm (length x diameter)
Number of holes: 31
diameter of side holes: 7.31 mm (Std.err = 0.09)
Arrangement of holes: Two sides of tube
Distance between holes: 6.73 mm (Std.err. = 0.04)
Soil type: Sandy-loam
Watering regime: 1 l once per week
Fertilizer application: Nil
Constraint: Breakage in soil column partly contributed to poor germination as well as the continuous breakage of the roots.

Seed source	sample	Taproot and lateral root characteristics						
		Length (mm)	Taproot diameter (mm)	Root collar diameter (cm)	Longest lateral (mm)	Lateral diameter i.e. 1cm from top of lateral (cm)	Distance of longest lateral from root collar (mm)	Distance from RCD to first lateral (mm)
Malawi	1	914	6.69	0.44	112	0.23	152	9
	2	709	4.82	0.28	107	0.12	90	12
	3	227	5.07	0.37	82	0.54	15	14
Namibia	1	663	4.72	0.39	18	0.08	612	19
	2	549	4.25	0.29	35	0.08	251	37
	3	652	8.13	0.25	738	0.55	56	19
South Africa	1	766	3.90	0.60	177	0.32	675	30
Tanzania	1	492	4.66	0.28	41	0.09	59	19
Tanzania	2	571	4.93	0.41	241	0.68	21	14
Mean		615.89	5.241	0.367	172.33	0.299	214.56	19.222
Standard Deviation		192.57	1.329	0.110	223.81	0.235	254.30	8.969
Std err.		64.19	0.443	0.037	74.60	0.078	84.77	2.989

Table 2.2.1. Soil chemical and physical characteristics used as potting medium for *P. angolensis*

Soil particle analysis and moisture characteristics

Depth (cm)	In %						Moisture (% v/v)		Available Moisture	
	Clay	Silt	Fine Sand	Medium Sand	Coarse Sand	Stone	10kPa	100kPa	Per sample	Per root depth
0-60	3.7	6.4	31.2	33.4	25.3	9.6	18.1	8.5	57.3mm/60cm =95.5mm/m	57.3mm/60cm

Soil chemical characteristics

Depth (cm)	pH (KCl)	Resistance (ohm)	H (cmol/kg)	Stone (Vol. %)	P (mg/kg)	K (mg/kg)
0	6.9	120	0	5	5880	5802

Table 2.2.2. Anova for the regression of the relationship between seedling age and shoot apical meristem characteristics as dependent variables.

SOV[a]	DF[a]	MEAN SQUARE						
		Tunica thickness	Corpus radius	SAM radius	SAM diameter	Tunica volume	Corpus volume	SAM volume
Regression	1	0.001	0.034	0.023	0.093	3.863	0.804	8.193
Residual	23	0.031	0.348	0.372	1.487	177	179	622
Lack of fit	3	0.019	0.325	0.403	1.610	190	113	579
Pure error	20	0.032	0.351	0.367	1.469	175	189	629
Adj. R^2		-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04
Parameter Estimates	Intercept (β_o)	0.775	1.689	2.464	4.927	23.22	14.55	37.78
	Coefficient (β_1)	-0.003	0.015	0.013	0.025	0.162	0.074	0.236

[a]/: SOV= source pf variation, DF= degrees of freedom
/: (^{NS})= not significant

Table 2.2.3a. Anova for the regression of the relationship between seedling age and cell number in tunica as predictors and shoot apical meristem characteristics as dependent variables.

SOV[a]	DF[a]		MEAN SQUARE					
		x	Seedling age	Cell number	Cell number	Cell number	Cell number	Cell number
		y	Cell number	Vertical distance	SAM diameter	Tunica thickness	Corpus radius	SAM radius
Regression	1		170*	1212718***	2255714***	11092^{NS}	385225***	857649***
Residual	15		36(32)	42758	116766	6271	20962	18818
Lack of fit	12		24.10(2)	29670	131423		19645(11)	18416
Pure error	3		37.13(12)	95108	58137		25788	20426
Adj. R^2			0.18	0.63	0.53		0.68	0.74
Parameter Estimates	Intercept (β_o)		32.51	-268	840		-959	75.86
	Coefficient (β_1)		-4.97	44	60		121	37.00
	Coefficient (β_2)		0.35				-2.24	

[a]/: SOV= source pf variation, DF= degrees of freedom
**/: (*)= significant at 5%, (**)= very significant at 1% , (***)= highly significant at 0.1% and (^{NS})= not significant*
(): Figures in parentheses are the respective degrees of freedom

Table 2.2.3b. Anova for the regression of the relationship between seedling age and cell number in tunica as predictors and shoot apical meristem characteristics as dependent variables.

SOV[a]	DF[a]		MEAN SQUARE				
		x	Cell number	Cell number	Cell number	Cell number	Cell number
		y	Corpus volume	Tunica volume	SAM volume	Primordium height	Primordium breadth
Regression	1		1.63E+18**	5.35E+18***	1.29E+19***	3003920**	2230600**
Residual	15		1.46E+17	1.39E+17	4.23E+17	174980(12)	188674(12)
Lack of fit	12		1.33E+17	1.48E+17	3.96E+17		
Pure error	3		1.98E+17	1.03E+17	5.03E+17		
Adj. R^2			0.39	0.70	0.65	0.55	0.45
Parameter Estimates	Intercept (β_o)		-584785367	-812665907	-1.40E+09	-496.704	-52.8
	Coefficient (β_1)		51008553	92419290	1.43E+08	68.705	59.2
	Coefficient (β_2)						

[a]/: SOV= source pf variation, DF= degrees of freedom
**/: (**)= very significant at 1% , (***)= highly significant at 0.1%*
(): Figures in parentheses are the respective degrees of freedom

Table 2.3.1a. Anova for testing differences in tissue type and seasonal effects on nutrient concentration in *P. angolensis*

SOV[a]	DF	MEAN SQUARES									
		N	P	K	Ca	Mg	Mn	Fe	Cu	Zn	B
Block	2	0.1802^{NS}	0.2555*	1.9142*	1.6507*	0.3370*	282.0702^{NS}	3739.1296^{NS}	14.8539^{NS}	160.5091^{NS}	725.7652*
Tissue	2	7.1299*	0.0025^{NS}	0.9475*	9.2917*	0.0058^{NS}	5569.8657*	126302.4630*	25.1617^{NS}	1777.2669*	6851.9230*
Season	3	0.4781*	0.0884*	1.3420*	0.2963^{NS}	0.0902*	713.0709^{NS}	45791.0679*	43.4378*	40.2068^{NS}	126.3060^{NS}
Residual	40	0.1512	0.0241	0.1856	0.1824	0.0151	381.7628	9755.7182	9.8543	58.6694	155.9363

[a]/: Abbreviations: SOV (source of variation), DF (degrees of freedom), N (nitrogen), P (Phosphorus), K (Potassium), Ca (Calcium), Mg (Magnesium), Mn (Manganese), Fe (Iron), Cu (Copper), Zn (Zinc), B (Boron).

*/: (*) is significant at p= 0.05, (**) is very significant at p= 0.01, (***) is highly significant at p=0.001) and (^{NS}) is not significant.

Table 2.3.1b. Anova for testing differences in seasonal effects for tissue types

SOV[a]	DF	FOLIAGE									
		MEAN SQUARE									
		N	P	K	Ca	Mg	Mn	Fe	Cu	Zn	B
Block	2	0.2494^{NS}	0.0960^{NS}	1.7526*	0.1366^{NS}	374761.7^{NS}	755.9306^{NS}	477.3889^{NS}	15.8872^{NS}	15.0839^{NS}	1752.9039*
Season	3	4.3508*	0.0149^{NS}	0.6072^{NS}	0.7676*	1318618.4*	818.1837^{NS}	3214.0926^{NS}	17.6004^{NS}	43.1948^{NS}	189.2181^{NS}
Residual	12	0.4709	0.0375	0.3361	0.1954	160300.1	1053.0611	2447.7824	8.8784	58.7555	365.4156

[a]/: Abbreviations: SOV (source of variation), DF (degrees of freedom), N (nitrogen), P (Phosphorus), K (Potassium), Ca (Calcium), Mg (Magnesium), Mn (Manganese), Fe (Iron), Cu (Copper), Zn (Zinc), B (Boron).

*/: (*) is significant at p= 0.05, (**) is very significant at p= 0.01, (***) is highly significant at p=0.001) and (^{NS}) is not significant.

Table 2.3.1c. Anova for testing differences in seasonal effects on nutrient concentration in the stem

SOV[a]	DF	STEM MEAN SQUARE									
		N	P	K	Ca	Mg	Mn	Fe	Cu	Zn	B
Block	2	0.5834^{NS}	0.1729^{*}	0.1249^{NS}	2.9400^{***}	0.3054^{***}	3.1272^{NS}	402.3889^{NS}	2.4956^{NS}	181.3506^{NS}	0.9217^{NS}
Season	3	0.2565^{NS}	0.0305^{NS}	0.2329^{NS}	0.0907^{NS}	0.0231^{NS}	146.2226^{NS}	10115.3704^{*}	30.6293^{*}	31.4626^{NS}	11.9300^{NS}
Residual	12	0.2747	0.0137	0.1098	0.0882	0.0084	124.0673	2575.6713	6.5337	62.6712	13.2043

[a]/: Abbreviations: SOV (source of variation), DF (degrees of freedom), N (nitrogen), P (Phosphorus), K (Potassium), Ca (Calcium), Mg (Magnesium), Mn (Manganese), Fe (Iron), Cu (Copper), Zn (Zinc), B (Boron).

**/: (*) is significant at p= 0.05, (**) is very significant at p= 0.01, (***) is highly significant at p=0.001) and (^{NS}) is not significant.*

Table 2.3.1d. Anova for testing differences in seasonal effects on nutrient concentration in the root

SOV[a]	DF	ROOT MEAN SQUARE									
		N	P	K	Ca	Mg	Mn	Fe	Cu	Zn	B
Block	2	0.1205^{NS}	0.0776^{*}	0.5548^{*}	0.0645^{NS}	0.1342^{*}	68.4306^{NS}	5592.3889^{NS}	3.2172^{NS}	87.2772^{NS}	87.2772^{NS}
Season	3	0.0536^{NS}	0.0844^{*}	0.6699^{*}	0.0259^{NS}	0.0450^{*}	199.1337^{NS}	72963.0370^{*}	25.8793^{NS}	139.7793^{NS}	139.7793^{*}
Residual	12	0.2019	0.0158	0.1372	0.0201	0.0021	82.7756	21792.6019	13.5705	39.3816	39.3816

[a]/: Abbreviations: SOV (source of variation), DF (degrees of freedom), N (nitrogen), P (Phosphorus), K (Potassium), Ca (Calcium), Mg (Magnesium), Mn (Manganese), Fe (Iron), Cu (Copper), Zn (Zinc), B (Boron).

**/: (*) is significant at p= 0.05, (**) is very significant at p= 0.01, (***) is highly significant at p=0.001) and (^{NS}) is not significant.*

Table 2.3.2. Seasonal and tissue nutrient concentration means

NUTRIENTS[a]	PHENOPHASE			TISSUE TYPE		
	Phenology	No.	Mean[b]	Tissue	No.	Mean[b]
Nitrogen (N)	Leaf Flush	9	1.88[a] (±0.26)	Foliage	15	2.43[a] (±0.15)
	Leaf Expansion	16	1.78[a] (±0.18)	Stem	15	1.32[b] (±0.06)
	Leaf Yellowing	9	1.65[a] (±0.25)	Root	18	1.21[b] (±0.09)
	Shoot Die-back	14	1.27[b] (±0.12)			
Phosphorus (P)	Leaf Flush	9	0.87[a] (±0.06)	Foliage	18	0.73[a] (±0.05)
	Leaf Expansion	18	0.76[ab] (±0.05)	Stem	18	0.75[a] (±0.04)
	Leaf Yellowing	9	0.64[b] (±0.04)	Root	18	0.75[a] (±0.04)
	Shoot Die-back	18	0.72[b] (±0.04)			
Potassium (K)	Leaf Flush	9	2.75[a] (±0.19)	Foliage	18	2.40[a] (±0.18)
	Leaf Expansion	18	2.04[b] (±0.12)	Stem	18	1.98[b] (±0.09)
	Leaf Yellowing	9	2.01[b] (±0.15)	Root	18	2.04[b] (±0.13)
	Shoot Die-back	18	2.01[b] (±0.14)			
Calcium (Ca)	Leaf Flush	9	1.12[a] (±0.24)	Foliage	18	1.76[a] (±0.13)
	Leaf Expansion	18	1.20[a] (±0.14)	Stem	18	1.65[a] (±0.15)
	Leaf Yellowing	9	1.34[a] (±0.29)	Root	18	0.47[b] (±0.04)
	Shoot Die-back	18	1.45[a] (±0.22)			
Magnesium (Mg)	Leaf Flush	9	0.83[a] (±0.06)	Foliage	18	0.67[a] (±0.04)
	Leaf Expansion	18	0.68[b] (±0.04)	Stem	18	0.68[a] (±0.05)
	Leaf Yellowing	9	0.68[b] (±0.06)	Root	18	0.71[a] (±0.04)
	Shoot Die-back	18	0.62[b] (±0.04)			
Manganese (Mn)	Leaf Flush	9	27.56[a] (±3.83)	Foliage	18	47.24[a] (±7.37)
	Leaf Expansion	18	34.01[a] (±7.84)	Stem	18	16.11[b] (±2.51)
	Leaf Yellowing	9	15.44[a] (±3.61)	Root	18	17.49[b] (±2.38)
	Shoot Die-back	18	25.33[a] (±5.26)			
Iron (Fe)	Leaf Flush	9	300[a] (±60.63)	Foliage	18	134[b] (±11.43)
	Leaf Expansion	18	165[b] (±26.28)	Stem	18	159[b] (±14.24)
	Leaf Yellowing	9	145[b] (±42.14)	Root	18	290[a] (±40.08)
	Shoot Die-back	18	196[b] (±18.49)			
Copper (Cu)	Leaf Flush	9	5.11[ab] (±1.44)	Foliage	18	6.67 (±0.79)
	Leaf Expansion	18	7.14[a] (±0.63)	Stem	18	4.54 (±0.76)
	Leaf Yellowing	9	2.78[b] (±0.47)	Root	18	6.49 (±0.90)
	Shoot Die-back	18	6.61[a] (±0.90)			
Zinc (Zn)	Leaf Flush	9	40.44[a] (±2.10)	Foliage	18	41.41[b] (±1.68)
	Leaf Expansion	18	42.64[a] (±2.86)	Stem	18	50.74[a] (±1.99)
	Leaf Yellowing	9	38.22[a] (±4.63)	Root	18	30.88[c] (±1.87)
	Shoot Die-back	18	41.06[a] (±2.68)			
Boron (B)	Leaf Flush	9	27.89[a] (±7.25)	Foliage	18	47.09[a] (±5.26)
	Leaf Expansion	18	26.46[a] (±5.17)	Stem	18	15.72[b] (±0.80)
	Leaf Yellowing	9	19.78[a] (±5.02)	Root	18	11.32[b] (±1.07)
	Shoot Die-back	18	23.83[a] (±5.28)			

[a]/: N, P, K, Ca and Mg are in % whilst Mn, Fe, Cu, Zn and B are in mg/kg

[b]/: Means followed by the same superscript are not significantly different

(): Standard errors for the means are in parentheses.

Table 2.3.3. Means for phenophasic nutrient concentration in foliage, stem and root of *P. angolensis*

TISSUE	PHENOPHASE	MINERAL NUTRIENT									
		Nitrogen (N)	Phosphorus (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Manganese (Mn)	Iron (Fe)	Copper (Cu)	Zinc (Zn)	Boron (B)
FOLIAGE	Leaf Flush	2.870[a] (±0.396)	0.76[a] (±0.11)	3.09[a] (±0.34)	1.13[a] (±0.26)	0.83[a] (±0.08)	36.33[a] (±18.74)	131.67[a] (±28.56)	5.67[a] (±1.72)	37.000[a] (±4.43)	53.33[a] (±11.04)
	Leaf Expansion	2.553[a] (±0.280)	0.79[a] (±0.08)	2.37[a] (±0.24)	1.60[a] (±0.18)	0.61[a] (±0.06)	60.73[a] (±13.25)	111.89[a] (±20.20)	8.18[a] (±1.22)	44.733[a] (±3.13)	50.61[a] (±7.80)
	Leaf Yellowing	2.497[a] (±0.396)	0.68[a] (±0.11)	2.20[a] (±0.34)	2.07[a] (±0.26)	0.78[a] (±0.08)	29[a] (±18.74)	119.33[a] (±28.56)	3.33[a] (±1.72)	40.667[a] (±4.43)	36.00[a] (±11.04)
	Shoot die-back	0.830[b] (±0.280)	0.69[a] (±0.08)	2.19[a] (±0.24)	2.09[a] (±0.18)	0.61[a] (±0.06)	48.33[a] (±13.25)	165.83[a] (±20.20)	7.33[a] (±1.22)	40.667[a] (±3.13)	46.00[a] (±7.80)
	All Phases	2.427 (±0.146)	0.73 (±0.05)	2.403 (±0.18)	1.76 (±0.13)	0.67 (±0.04)	47.25 (±7.37)	134 (±11.43)	6.67 (±0.79)	41.41 (±1.68)	47.09 (±5.26)
STEM	Leaf Flush	1.403[a] (±0.304)	0.86[a] (±0.07)	2.38[a] (±0.19)	1.68[a] (±0.17)	0.80[a] (±0.05)	19.33[a] (±6.43)	237[a] (±29.30	0.33[b] (±1.48)	45.667[a] (±4.57)	16.67[a] (±2.10)
	Leaf Expansion	0.950[a] (±0.214)	0.75[a] (±0.05)	1.79[a] (±0.14)	1.50[a] (±0.12)	0.69[a] (±0.04)	21.65[a] (±4.55)	122[b] (±20.71)	6.28[a] (±1.04)	52.050[a] (±3.23)	17.31[a] (±1.48)
	Leaf Yellowing	1.383[a] (±0.302)	0.62[a] (±0.07)	1.94[a] (±0.19)	1.63[a] (±0.17)	0.63[a] (±0.05)	9.33[a] (±6.43)	128[b] (±29.30)	2.67[ab] (±1.48)	52.000[a] (±4.57)	14.67[a] (±2.10)
	Shoot die-back	0.960[a] (±0.214)	0.77[a] (±0.05)	1.99[a] (±0.14)	1.80[a] (±0.12)	0.62[a] (±0.04)	12.33[a] (±4.55)	171[ab] (±20.72)	5.83[a] (±1.04)	51.333[a] (±3.23)	14.17[a] (±1.48)
	All Phases	1.321 (±0.064)	0.75 (±0.04)	1.98 (±0.09)	1.65 (±0.15)	0.68 (±0.05)	16.11 (±2.51)	159 (±14.24)	4.54 (±0.76)	50.74 (±1.99)	15.72 (±0.80)
ROOT	Leaf Flush	1.370[a] (±0.259)	1[a] (±0.07)	2.77[a] (±0.21)	0.55[a] (±0.08)	0.87[a] (±0.03)	27[a] (±5.25)	530[a] (±85.23)	9.33[a] (±2.13)	38.667[a] (±3.62)	13.67[a] (±2.74)
	Leaf Expansion	1.242[a] (±0.183)	0.74[b] (±0.05)	1.96[b] (±0.15)	0.50[a] (0.06)	0.73[b] (±0.02)	19.65[a] (±3.71)	261[b] (±60.27)	6.97[a] (±1.50)	31.133[ab] (±2.56)	11.44[a] (±1.93)
	Leaf Yellowing	1.060[a] (±0.259)	0.62[b] (±0.07)	1.88[b] (0.21)	0.34[a] (±0.08)	0.61[c] (±0.03)	8[a] (±5.25)	187[b] (±85.23)	2.33[a] (±2.13)	22.000[b] (±3.62)	8.67[a] (±2.74)
	Shoot die-back	1.168[a] (±0.183)	0.70[b] (±0.05)	1.82[b] (±0.15)	0.46[a] (±0.06)	0.65[c] (±0.02)	15.33[a] (±3.71)	251[b] (±60.27)	6.67[a] (±1.50)	31.167[ab] (±2.56)	11.33[a] (1.93)
	All Phases	1.208 (±0.092)	0.75 (±0.04)	2.04 (±0.13)	0.47 (±0.04)	0.71 (±0.04)	17.50 (±2.38)	290 (±40.08)	6.49 (±0.90)	30.88 (±1.87)	11.32 (±1.07)

[a]/: *N, P, K, Ca and Mg are in % whilst Mn, Fe, Cu, Zn and B are in mg/kg*
[b]/: *Means followed by the same superscript are not significantly different*
(): Standard errors for the means are in parentheses.

Table 2.3.4a. Correlations in mineral nutrient concentrations on a whole plant basis showing seasonal changes in correlations between nutrients

Mineral Element	Phenophase	Mineral Element				
		N	P	K	Ca	Mg
P	Leaf flush	-0.611				
	Leaf expansion	0.264				
	Leaf yellowing	0.437				
	Shoot die-back	0.199				
K	Leaf flush	0.520	-0.100			
	Leaf expansion	0.526	0.701			
	Leaf yellowing	0.479	0.729			
	Shoot die-back	0.301	0.391			
Ca	Leaf flush	0.461	-0.407	-0.334		
	Leaf expansion	0.567	0.283	0.367		
	Leaf yellowing	0.593	0.208	-0.116		
	Shoot die-back	0.274	-0.004	-0.382		
Mg	Leaf flush	0.055	0.408	-0.252	0.029	
	Leaf expansion	-0.184	0.462	-0.128	-0.159	
	Leaf yellowing	0.638	0.567	0.061	0.720	
	Shoot die-back	0.050	0.194	-0.451	0.471	
Mn	Leaf flush	0.337	-0.019	-0.202	0.477	0.707
	Leaf expansion	0.478	0.557	0.722	0.582	-0.207
	Leaf yellowing	0.862	0.349	0.349	0.603	0.603
	Shoot die-back	0.627	-0.079	-0.106	0.524	0.437

NB. Correlations in bold type are significant.

Table 2.3.4b. Correlations in mineral nutrient concentrations on a whole plant basis showing seasonal changes in correlations between nutrients

Mineral Element	Phenophase	Mineral Element								
		N	P	K	Ca	Mg	Mn	Fe	Cu	Zn
Fe	Leaf flush	-0.741	0.758	-0.181	-0.769	0.271	-0.249			
	Leaf expansion	-0.241	0.412	0.386	-0.353	0.326	0.154			
	Leaf yellowing	-0.125	0.317	0.439	-0.469	-0.149	0.046			
	Shoot die-back	-0.202	0.191	0.152	-0.518	-0.147	-0.040			
Cu	Leaf flush	-0.046	0.335	0.432	-0.826	0.290	-0.270	0.637		
	Leaf expansion	0.575	0.525	0.684	0.167	0.113	0.444	0.540		
	Leaf yellowing	0.045	0.373	0.497	-0.205	-0.007	0.390	0.795		
	Shoot die-back	0.636	0.474	0.621	-0.408	-0.138	0.071	0.113		
Zn	Leaf flush	-0.397	0.622	-0.476	0.381	0.228	0.304	0.194	-0.459	
	Leaf expansion	0.419	0.398	0.320	0.599	0.079	0.181	-0.247	0.248	
	Leaf yellowing	0.259	0.101	0.069	0.598	0.298	0.114	-0.118	-0.143	
	Shoot die-back	0.072	0.237	0.071	0.588	0.263	-0.095	-0.388	-0.129	
B	Leaf flush	0.739	-0.290	0.029	0.155	0.345	0.714	-0.709	-0.475	0.253
	Leaf expansion	0.652	0.473	0.546	0.658	-0.214	0.866	-0.118	0.405	0.213
	Leaf yellowing	0.477	0.185	0.024	0.715	0.506	0.765	-0.271	0.307	0.075
	Shoot die-back	0.670	-0.244	-0.296	0.332	0.165	0.723	-0.398	0.091	-0.053

NB. Correlations in bold type are significant.

Table 2.3.5. Anova for the regression and test of lack of fit of the relationship between mineral nutrients with significant seasonal correlations

		MEAN SQUARE					
		Leaf yellowing & shoot die-back	Expansion & Shoot die-back	Leaf expansion & leaf yellowing	Leaf expansion & shoot die-back	Leaf expansion & yellowing	Leaf expansion, yellowing & shoot die-back
SOV[a]	DF[a]	x=N, y=Mn	x=B, y=N	x=K, y=P	x=K, y=Cu	x=Ca, y=B	X=Mn, y=B
Regression	1	3.291^{***}	4833.803^{***}	2.541^{***}	4.879^{***}	4.353^{***}	$129899.340(3)^{***}$
Residual[b]	21	0.221	196.266(28)	0.145(25)	0.154(34)	0.302(25)	147.110(42)
Lack of fit[b]	16	0.219^{NS}	$209.721(26)^{NS}$	$0.147(23)^{NS}$	$0.143(19)^{NS}$	$0.343(21)^{NS}$	
Pure error[b]	5	0.225^{*}	$21.348(2)^{**}$	$0.133(2)^{*}$	$0.169(15)^{***}$	$0.086(4)^{**}$	
Adj. R^2		0.415	0.449	0.388	0.466	0.340	
Parameter Estimates	Intercept (β_o)	0.794	-8.824	0.830	1.232	0.746	0.295^{NS}
	Coefficient (β_1)	0.036	19.971	1.666	0.115	0.021	1.128^{***}
	Coefficient (β_2)						0.0047^{*}

[a]/: SV= source of variation, DF= degrees of freedom for residual

[b]/: () number in parenthesis is DF

**/: (*) is significant at p=0.05), (**) is significant at p=1%, (***) is significant at p=0.1%) and (^{NS}) is not significant*

Table 2.4.1. Planting site and potting soil's chemical and physical characteristics

	Depth (cm)	Soil type	pH (KCl)	Resist. (Ohm)	H (cmol/kg)	Stone (Vol %)	P Bray II	K	Exchangeable cations (cmol(+)/kg)				Base saturation				T-value (cmol/kg)
							mg/kg	mg/kg	Na	K	Ca	Mg	Na %	K %	Ca %	Mg %	
Planting Site	0	Sand	3.8	5900	1.54	7	5	10	0.00	0.02	0.34	0.22	0.05	1.17	15.99	10.25	2.12
Potting soil	0	sand	4.7	3320	1.35	24	11	9	0.06	0.02	5.24	4.36	0.53	0.21	47.50	39.52	11.02

Table 2.4.2. Planting plan for the Sudwala Caves field experiment

ROAD

Block 4

R17			R7			R14		
N2	S4	N3	S1	T3	T4	S4	N2	N3
S3	N1	T1	S4	N1	T2	S3	N4	T4
S2	N4	S1	T1	Z2	T3	T2	T3	N1
T2	T3	T4	N4	S3	N2	S1	T1	Z2

Block 3

R16			R6			R12		
N3	N2	S2	T2	T4	S4	T3	T4	N4
N1	T3	T2	N4	S3	S2	N2	S4	N3
T1	T4	N4	N1	S1	N2	T2	N1	T1
S1	S3	S4	N3	T1	T3	S3	S1	S2

R4			R8		
N1	S1	N4	S1	T4	N1
T1	S3	S4	S3	N2	S4
T4	S2	N2	N3	N4	T2
T3	N3	T2	S2	T3	T1

Block 2

R10			R3			R15		
S4	N3	T1	T3	N1	N2	S3	S1	N4
T4	S1	S3	S4	T4	S1	N3	N1	N2
T3	N2	S2	T2	T1	N3	S2	T4	T3
T2	N1	N4	N4	S3	S2	T1	T2	S4

Block 1

R9				R13				R11			
T1	N4	N3	S4	N1	S2	S3	T2	N2	S3	S2	T3
N1	S1	T4	T2	T4	N4	S4	N2	S4	N3	N1	N4
T3	S2	N2	S3	S1	N3	T1	T3	T4	S1	T2	T1

R5				R2				R1			
T1	T2	N1	S2	T4	S1	T2	T1	T3	S2	N1	S4
N2	S1	N3	N4	N2	T3	N1	N4	T4	T1	S3	N2
T4	S4	T3	S3	S3	S4	N3	S2	N3	S1	N4	T2

Explanation of designations:

Date of estabishment - 19/12/2001

Suffixes 1, 2 and 3 indicate seedlings of ages 1, 2 and 3 months.

Suffixe 4 indicates direct sowing

N= Namibian seed source S= South African seed source T= Tanzanian seed source

Boxed combinations indicate replanting (beating up) carried out on 05/01/2001

Table 2.4.3a. Anova for testing differences in seedling ages at planting and provenance on seedling survival and height in P. angolensis for June 2001 and January 2002

SOV	DF	MEAN SQUARES[§]			
		Survival		Seedling height	
		June 2001	Jan 2002	June 2001	Jan 2002
Replication	13	0.823	0.818^{NS}	21.68	15.32
Provenance	2	0.543^{NS}	0.397^{NS}	427.84^{***}	4.19^{NS}
Seedling age at planting	3	7.226^{***}	4.876^{***}	33.22^{NS}	4.34^{NS}
Residual	483	0.452	0.432	18.23	13.38

[a]/: SOV= source of variation, DF= degrees of freedom
[b]/: (***) = significant at p=0.1%, (^{NS}) = not significant

Table 2.4.3b. Comparison of means for seedling survival, mortality and height for June 2001 and January 2002

Variable		MEANS			
		Survival (%)		Height (in cm)	
		June 2001	Jan 2002	June 2001	Jan 2002
Seed source (provenance)	Namibia	41.07^{a}	26.79^{a}	6.90^{b}	9.35^{a}
	South Africa	35.71^{a}	37.50^{a}	5.83^{b}	8.94^{a}
	Tanzania	48.21^{a}	32.14^{a}	13.67^{a}	9.87^{a}
Glasshouse seedling age at planting	Direct sowing	2.38^{b}	0.00^{b}	1.50^{a}	-
	1	52.38^{a}	40.48^{a}	7.93^{a}	10.42^{a}
	2	59.52^{a}	47.62^{a}	8.38^{a}	8.67^{a}
	3	52.38^{a}	40.48^{a}	10.20^{a}	9.14^{a}

Means followed by the same superscript are not significantly different.

Table 2.4.4a. Anova for testing interaction effects between seedling age at planting, provenance and time of assessment on seedling survival

SOV[a]	DF[a]	Survival
		MEAN SQUARE[b]
Replication	13	2.158
Provenance	2	0.739^{NS}
Seedling age at planting (1)	3	14.431^{***}
Time of assessment (2)	2	3.530^{***}
Interaction (1*2)	6	0.390^{NS}
Residual	477	0.3981

[a]/: SOV= source of variation, DF= degrees of freedom
[b]/: (***) = significant at p=0.1%, (^{NS}) = not significant

Table 2.4.4b. Comparison of means for seedling survival for assessment made in June 2001, January 2002 and August 2002

VARIABLE	MEANS						
	Seedling age at planting				Time of assessment		
	Direct sowing	1	2	3	Jul. 2001	Jan. 2002	Aug. 2002
Survival (in %)	0.79[b]	38.10[a]	49.21[a]	41.27[a]	41.67[a]	32.14[b]	23.21[b]

Table 2.4.5a. Anova for assessing the effect of seedling age at planting and provenance on the occurrence of shoot die-back in three seed sources (August 2002)

SOV[a]	DF[a]	MEAN SQUARES[b]				
		Shoot die-back	Live leafless Stem	Seedling with leaves	Live stem with dead leaves	Mortality (seedling is absent)
Replication	13	0.253	0.185	0.105	0.027	0.828
Provenance	2	0.103^{NS}	0.308^{NS}	0.024^{NS}	0.059^{NS}	0.044^{NS}
Seedling age at planting	3	0.372^{NS}	0.509^{*}	0.149^{NS}	0.020^{NS}	3.109^{***}
Residual	149	0.182	0.154	0.057	0.029	0.361

[a]/: SOV= source of variation, DF= degrees of freedom
[b]/: (*) = significant at 5%, (***) = significant at p=0.1%

Table 2.4.5b. Comparison of means of the effect of seedling age at planting on leaf loss and seedling mortality in field grown P. angolensis seedlings (August 2002)

VARIABLE	SEEDLING AGE AT PLANTING (in months)			
	Direct sowing	1	2	3
Live leafless Stem (%)	0.0^{b}	4.76^{ab}	16.67^{a}	7.14^{ab}
Mortality (%)	100^{a}	78.57^{ab}	59.52^{b}	69.05^{b}

Means followed by the same superscript are not significantly different.

Table 3.1. Effect of water treatments on Chl. a fluorescence in dark-adapted leaves

Treatment	F_0	F_M	F_0/F_M	F_V/F_0	V_J	V_I	$(dV/dt)_0$	S_M/t_{Fmax}
30%	516(55)	1570(260)	0.33(0.06)	2.06(0.55)	0.60(0.04)	0.88(0.03)	1.56(0.17)	0.05(0.02)
40%	546(56)	1752(198)	0.32(0.05)	2.24(0.56)	0.58(0.04)	0.87(0.04)	1.34(0.23)	0.06(0.03)
60%	489(23)	1701(136)	0.29(0.03)	2.50(0.43)	0.57(0.05)	0.88(0.03)	1.26(0.21)	0.06(0.02)
80%	477(30)	1807(131)	0.26(0.01)	2.79(0.09)	0.55(0.03)	0.85(0.03)	1.31(0.17)	0.06(0.02)
100%	517(61)	1697(158)	0.31(0.04)	2.32(0.51)	0.55(0.05)	0.87(0.03)	1.23(0.21)	0.05(0.01)
F	1.66	1.07	1.84	1.69	1.05	0.64	1.79	0.22
p	0.20	0.39	0.16	0.19	0.41	0.64	0.17	0.92

Treatment	S_M	N	Area	ABS/RC	TR_0/RC	ET_0/RC	Φ_{Po}	Φ_{Eo}
30%	18(3.9)	46(13)	18 570(5426)	3.9(0.6)	2.61(0.3)	1.04(0.19)	0.67(0.06)	0.27(0.03)
40%	18(3.5)	41(4)	21 600(2559)	3.4(0.4)	2.3(0.3)	0.96(0.10)	0.68(0.05)	0.29(0.03)
60%	16(1.5)	35(4)	19 387(3530)	3.1(0.4)	2.2(0.2)	0.92(0.08)	0.71(0.03)	0.30(0.05)
80%	20(3.0)	47(4)	26 780(6041))	3.2(0.4)	2.4(0.3)	1.05(0.14)	0.74(0.01)	0.33(0.02)
100%	20(3.0)	45(8)	23 960(6013)	3.2(0.7)	2.2(0.3)	1.00(0.13)	0.69(0.04)	0.31(0.04)
F	1.68	2.23	2.24	2.09	1.60	0.88	1.84	1.80
P	0.20	0.10	0.10	0.12	0.22	0.50	0.16	0.17

NB: Number in parenthesis is the standard deviation.

continued

Table 3.1 (continued)

Treatment	Ψ_o	RC/CS_o	RC/CS_M	ABS/CS_0	ABS/CS_M	ET_o/CS_0	TR_0/CS_0	TR_0/CS_M
30%	0.40(0.04)	133(18)	412(123)	516(55)	1570(260)	136(16)	342(39)	1054(253)
40%	0.42(0.04)	163(15)	526(84)	546(56)	1752(198)	156(22)	372(24)	1206(218)
60%	0.43(0.05)	160(18)	567(134)	489(23)	1701(136)	148(18)	347(8)	1213(154)
80%	0.45(0.03)	151(26)	572(109)	477(30)	1807(131)	157(17)	351(23)	1330(103)
100%	0.45(0.05)	162(20)	542(132)	517(61)	1697(158)	162(29)	357(30)	1180(164)
F	1.05	1.77	1.31	1.66	1.07	0.99	0.92	1.31
P	0.41	0.18	0.30	0.20	0.40	0.44	0.47	0.30

Treatment	K_N	K_P	$SFI_{(ABS)}$	$PI_{(ABS)}$	$PI_{(CSo)}$	$PI_{(CSm)}$	$DF_{(ABS)}$
30%	0.65(0.11)	1.30(0.22)	0.69(0.18)	3.66(1.56)	1847(652)	5919(3024)	1.23(0.44)
40%	0.58(0.06)	1.27(0.21)	0.87(0.18)	4.98(2.01)	2669(866)	8912(4418)	1.55(0.34)
60%	0.59(0.05)	1.46(0.14)	1.02(0.35)	6.72(4.15)	3213(1786)	11 819(8358)	1.79(0.50)
80%	0.56(0.04)	1.55(0.09)	1.03(0.16)	7.11(1.39)	3418(836)	12 987(3397)	1.95(0.19)
100%	0.59(0.06)	1.36(0.24)	1.00(0.28)	6.36(2.91)	3221(1324)	11 097(5769)	1.76(0.47)
F	1.27	1.81	1.54	1.28	1.25	1.16	2.07
P	0.32	0.17	0.23	0.31	0.33	0.36	0.12

NB: Number in parenthesis is the standard deviation.

Table 3.2. Effect of water treatments on Chl. a fluorescence in light-adapted leaves

Treatment	F_0	F_M	F_0/F_M	F_V/F_0	V_J	V_I	$(dV/dt)_0$	S_M/t_{Fmax}
30%	694(36)	902(59)	0.77(0.09)	0.31(0.14)	0.70(0.04)	0.97(0.01)	1.76(0.14)	0.02(0.003)
40%	829(106)	1081(142)	0.77(0.05)	0.31(0.09)	0.71(0.07)	0.96(0.02)	1.74(0.45)	0.02(0.009)
60%	773(88)	977(73)	0.79(0.06)	0.27(0.10)	0.70(0.03)	0.96(0.01)	1.61(0.25)	0.02(0.007)
80%	778(59)	1019(44)	0.76(0.05)	0.32(0.09)	0.69(0.04)	0.96(0.01)	1.63(0.28)	0.02(0.005)
100%	758(94)	939(84)	0.81(0.07)	0.25(0.11)	0.67(0.03)	0.96(0.01)	1.51(0.25)	0.02(0.008)
F	1.52	2.89	0.39	0.39	0.40	0.38	0.56	0.35
P	0.24	0.05	0.81	0.82	0.81	0.82	0.69	0.84

Treatment	S_M	N	Area	ABS/RC	TR_0/RC	ET_0/RC	Φ_{Po}	Φ_{Eo}
30%	8(2.9)	21(8)	1636(803)	13(6.7)	2.59(0.17)	0.75(0.12)	0.23(0.09)	0.07(0.03)
40%	7(2.7)	16(5)	11645(569)	11(3.7)	2.43(0.36)	0.69(0.11)	0.24(0.05)	0.07(0.03)
60%	12(11)	28(28)	2255(1696)	12(4.7)	2.30(0.25)	0.69(0.05)	0.21(0.06)	0.06(0.02)
80%	9(2)	22(7)	2297(968)	11(3.9)	2.33(0.30)	0.71(0.03)	0.24(0.05)	0.07(0.02)
100%	7(2)	15(5)	1120(292)	14(7.2)	2.23(0.27)	0.72(0.04)	0.19(0.07)	0.06(0.03)
F	0.74	0.70	1.21	0.30	0.69	0.45	0.39	0.13
P	0.57	0.60	0.34	0.87	0.61	0.77	0.81	0.97

NB: Number in parenthesis is the standard deviation.

continued

Table 3.2 (continued)

Treatment	Ψ_o	RC/CS_o	RC/CS_M	ABS/CS_0	ABS/CS_M	ET_o/CS_0	TR_0/CS_0	TR_0/CS_M
30%	0.30(0.04)	63(25)	85(42)	694(36)	902(59)	47(19)	155(54)	208(126)
40%	0.29(0.07)	80(21)	105(35)	829(106)	1081(142)	56(19)	191(46)	252(100)
60%	0.30(0.03)	71(23)	92(37)	773(88)	977(73)	49(15)	159(35)	204(69)
80%	0.31(0.04)	80(23)	106(36)	778(59)	1019(44)	56(16)	182(34)	242(46)
100%	0.33(0.03)	64(24)	82(35)	758(94)	939(84)	47(17)	142(49)	181(73)
F	0.40	0.57	0.46	1.52	2.89	0.37	1.04	0.79
P	0.81	0.69	0.77	0.24	0.05	0.83	0.41	0.55

Treament	$\frac{\Delta F}{F_M^L}$ PFD x (Relative electron transport rate)	$1-\left(\frac{F_M^L - F_0^L}{F_M^L - \Phi_{Po}^{dark}}\right)$ (Quantum yield limitation)
30%	18(7.0)	0.67(0.11)
40%	19(4.2)	0.66(0.06)
60%	17(4.8)	0.71(0.07)
80%	19(4.3)	0.68(0.07)
100%	15(6.0)	0.73(0.10)
F	0.39	0.51
p	0.81	0.73

NB: Number in parenthesis is the standard deviation.

continued

Table 3.2 (continued)

Treatment	K_N	K_P	$SFI_{(ABS)}$	$PI_{(ABS)}$	$PI_{(CSo)}$	$PI_{(CSm)}$	$DF_{(ABS)}$
30%	1.11(0.07)	0.33(0.14)	0.07(0.05)	0.14(0.11)	97(71)	134(105)	-2.32(1.18)
40%	0.94(0.13)	0.29(0.05)	0.07(0.04)	0.14(0.08)	113(68)	153(98)	-2.19(0.89)
60%	1.03(0.08)	0.28(0.10)	0.07(0.05)	0.13(0.10)	97(72)	129(103)	-2.32(0.92)
80%	0.98(0.04)	0.31(0.08)	0.08(0.05)	0.16(0.11)	126(86)	170(125)	-2.02(0.77)
100%	1.07(0.10)	0.26(0.11)	0.06(0.04)	0.12(0.09)	89(64)	117(86)	-2.46(1.08)
F	2.74	0.33	0.12	0.12	0.20	0.20	0.15
P	0.06	0.85	0.97	0.97	0.94	0.93	0.96

NB: Number in parenthesis is the standard deviation.

Table 3.3. Effect of water treatments on Chl. a fluorescence for the difference between dark-adapted and light-adapted leaves

Treatment	F_0	F_M	F_0/F_M	F_V/F_0	V_J	V_I	$(dV/dt)_0$	S_M/t_{Fmax}
30%	-178(68)	668(213)	-0.44(0.03)	1.76(0.41)	-0.10(0.03)	-0.09(0.03)	-0.20(0.19)	0.03(0.03)
40%	-283(132)	671(250)	-0.45(0.04)	1.94(0.50)	-0.13(0.06)	-0.08(0.03)	-0.41(0.41)	-0.04(0.02)
60%	-284(77)	724(125)	-0.50(0.04)	2.23(0.35)	-0.12(0.04)	-0.08(0.03)	-0.35(0.16)	0.04(0.02)
80%	-300(76)	788(134)	-0.50(0.05)	2.47(0.12)	-0.14(0.04)	-0.10(0.03)	-0.31(0.12)	0.05(0.02)
100%	-241(51)	758(168)	-0.50(0.07)	2.08(0.47)	-0.12(0.03)	-0.09(0.02)	-0.28(0.06)	0.03(0.01)
F	1.43	0.40	1.84	2.25	1.05	0.69	0.52	0.44
P	0.26	0.81	0.16	0.10	0.41	0.61	0.72	0.78

Treatment	S_M	N	Area	ABS/RC	TR_0/RC	ET_0/RC	Φ_{Po}	Φ_{Eo}
30%	9(2.7)	25(12)	16934(5057)	-9(6)	0.09(0.20)	0.29(0.09)	0.43(0.03)	0.20(0.03)
40%	11(1.0)	25(3)	19955(2240)	-7(3)	-0.14(0.41)	0.27(0.13)	0.45(0.04)	0.22(0.06)
60%	4(2.8)	7(25)	17132(3878)	-9(4)	-0.12(0.14)	0.23(0.05)	0.50(0.04)	0.24(0.04)
80%	11(1.9)	25(9)	24482(6027)	-71(3)	0.03(0.12)	0.35(0.13)	0.50(0.05)	0.25(0.04)
100%	13(1.1)	30(7)	22840(5879)	-11(7)	-0.004(0.11)	0.28(0.11)	0.50(0.07)	0.25(0.03)
F	1.83	2.09	2.32	0.30	0.89	0.76	1.85	2.59
P	0.16	0.12	0.09	0.87	0.49	0.57	0.16	0.07

NB: Number in parenthesis is the standard deviation.

continued

Table 3.3 (continued)

Treatment	Ψ_o	RC/CS_o	RC/CS_M	ABS/CS_0	ABS/CS_M	ET_o/CS_0	TR_0/CS_0	TR_0/CS_M
30%	-0.44(0.03)	70(16)	326(83)	-178(68)	668(213)	89(12)	187(43)	846(164)
40%	-0.45(0.04)	84(22)	421(59)	-283(132)	671(250)	101(25)	181(62)	954(191)
60%	-0.50(0.04)	90(12)	475(103)	-284(77)	724(125)	99(12)	188(34)	1009(102)
80%	-0.50(0.05)	71(19)	466(89)	-301(76)	788(134)	100(13)	169(33)	1089(100)
100%	-0.50(0.07)	97(30)	460(121)	-241(51)	758(168)	115(36)	215(72)	1000(176)
F	0.44	1.53	1.81	1.44	0.40	0.79	0.53	1.54
P	0.77	0.23	0.17	0.26	0.81	0.55	0.71	0.23

Treatment	K_N	K_P	$SFI_{(ABS)}$	$PI_{(ABS)}$	$PI_{(CSo)}$	$PI_{(CSm)}$	$DF_{(ABS)}$
30%	-0.46(0.06)	0.97(0.17)	0.62(0.15)	3.55(0.84)	1749(589)	5785(2930)	3.55(0.84)
40%	-0.36(0.15)	0.98(0.19)	0.79(0.16)	3.75(0.75)	2556(829)	8759(4360)	3.75(0.75)
60%	-0.44(0.07)	1.18(0.09)	0.95(0.31)	4.11(0.61)	3116(1729)	11690(8276)	4.12(0.611)
80%	-0.43(0.06)	1.24(0.15)	0.95(0.12)	3.96(0.62)	3292(769)	12817(3299)	3.96(0.62)
100%	-0.48(0.10)	1.10(0.18)	0.94(0.26)	4.22(0.95)	3131(1291)	10980(5717)	4.22(0.95)
F	1.04	2.65	1.98	1.33	1.34	1.18	0.58
P	0.41	0.07	0.14	0.29	0.29	0.35	0.68

NB: Number in parenthesis is the standard deviation.

Table 4.1. Analysis of variance, variance component and narrow sense heritability estimation for shoot die-back of 16 half-sib families of *P. angolensis* seedlings

sov[a]	df[a]	MEAN SQUARE		VARIANCE COMPONENT ESTIMATE	
		2001[b]	2002[b]	2001(±SE)	2002(±SE)
Replication (R)	5	1.82	0.59		
Provenance (P)	5	0.12*	4.36*		
R*P	25	0.85	0.47	0.599	
Family(Provenance)	10	1.04NS	1.69***	0.028(±0.025)[d]	0.082(±0.047)[d]
Residual	215	0.55(253)[c]	0.49	0.549	0.493
h^2				0.07(±0.07)[e]	0.42(±0.27)[e]

[a]/: *SOV=source of variation, DF=degrees of freedom*
[b]/: *NS=not significant; ***=highly significant at 0.1%*
[C]/: *Number in parenthesis is error degrees of freedom*
[d]/:*Number in parenthesis is standard error of the estimate of variance component*
[e]/:*Number in parenthesis is standard error of the heritability*

Table 4.2. Comparison of half-sib family means (in per cent) for shoot die-back for 2001 and 2002

HALF-SIB FAMILY*	2001	HALF-SIB FAMILY*	2002
M74	71[a](±18)	N87	91[a](±9)
N10	60[a](±10)	M82	81[ab](±10)
M32	56[a](±12)	M19	80[ab](±13)
M87	50[a](±17)	N5	69[abc](±9)
N5	48[a](±10)	Z5	67[abc](±14)
M19	47[a](±13)	Z16	67[abc](±14)
M119	47[a](±13)	M70	56[abcd](±12)
Z16	47[a](±13)	M119	53[abcd](±13)
M82	45[a](±11)	Z19	41[abcd](±12)
M125	43[a](±11)	M74	40[abcd](±25)
Z19	40[a](±11)	M29	33[bcd](±11)
M29	35[a](±12)	N8	29[bcd](±10)
M70	30[a](±11)	N10	24[cd](±10)
N8	30[a](±10)	M32	23[cd](±12)
N7	29[a](±9)	N7	15[cd](±7)
Z5	20[b](±13)	M125	10[d](±7)
All Families	42(±3)	All Families	45(±3)

**/: Prefix M = Malawian, N = Namibian and Z=Zambian half-sib family*

[a]/: Means followed by the same letter(s) are not significantly different

(): Numbers in parentheses are standard errors

Table 4.3. Anova for half-sib family variation in shoot height, taproot length, root collar diameter and biomass of *P. angolensis* seedlings

sov[a]	df[a]	MEAN SQUARE[B]					
		Shoot height	Taproot length	Root collar diameter	Shoot biomass	Root biomass	Shoot:Root biomass
Replication (R.)	5	0.007	0.004	0.003	55	1.66	142
Provenance (P)	5	0.020^{NS}	0.002^{NS}	0.019^{***}	1010^{***}	2.65^{***}	289^{***}
R*P	25	0.008	0.004	0.001	516	0.46	97
Family(provenance)	10	0.030***	0.0005^{NS}	0.005***	561**	2.18***	216*
Residual[c]	157	0.009	0.002(165)	0.0007(165)	195(157)	0.42(163)	91(155)

[a]/: SOV= source of variation, DF=degrees of freedom
[b]/: (*)=significant at 5%; (**)= very significant at 1%; (***)= highly significant at 0.1%; (^{NS})= not significant
[c]/: Number in parenthesis is the error degrees of freedom

Table 4.4. Variance component and narrow sense heritability estimates for shoot height, shoot biomass, root collar diameter, taproot length, root biomass and the ratio between shoot and root biomass

sov[a]	VARIANCE COMPONENT AND NARROW SENSE HERITABILITY ESTIMATES[B]				
	Shoot height	RCD[a]	Shoot biomass	Root biomass	Sdw/Rdw[a]
Replication (R)	0.0001	0.00005		0.043	0.58
Provenance (P)					
R*P		0.00003	67	0.010	1.18
Family(provenance)	0.002(0.001)	0.0003(0.00016)	33(21)	0.157(0.079)	11.53(8.23)
Residual	0.009	0.0007	196	0.416	91.37
h^2	0.54(0.35)	0.90(0.51)	0.33(0.24)	0.81(0.45)	0.33(0.26)

[a]/: SOV= source of variation, RCD=root collar diameter, $\frac{Sdw}{Rdw}$ *=ratio of shoot to root biomass*
():Number in parenthesis is the standard error of the variance component or h^2

Table 4.5. Comparison of means for half-sib family variation in shoot and root parameters of *P. angolensis* seedlings

Family	Shoot height (cm)	Family	Rcd* (mm)	Family	Taproot length (cm)	Family	Sdw* (g)	Family	Rdw* (g)	Family	Sdw/rdw
M29	13.6^{b}(±1.15)	M29	9.9^{a}(±0.44)	M125	18.8^{a}(±1.50)	M29	1.04^{a}(±0.16)	M29	2.39^{a}(±0.27)	M29	0.47^{a}(±0.08)
Z19	12.0^{ab}(±1.73)	M125	9.8^{a}(±0.52)	Z16	18.6^{a}(±0.56)	M125	0.73^{b}(±0.12)	M125	2.23^{a}(±0.29)	N10	0.45^{a}(±0.04)
M125	10.7^{ab}(±1.01)	Z16	8.9^{ab}(±0.42)	Z5	18.1^{a}(±2.84)	N10	0.56^{bc}(±0.10)	Z16	1.55^{b}(±0.20)	Z19	0.39^{ab}(±0.08)
Z16	10.1^{ab}(±1.40)	Z19	8.6^{ab}(±0.35)	N7	17.8^{a}(±1.76)	Z19	0.54^{bc}(±0.10)	M70	1.48^{b}(±0.25)	N5	0.38^{ab}(±0.03)
N8	9.7^{ab}(±0.57)	M87	7.7^{abc}(±0.55)	M87	17.4^{a}(±2.21)	Z16	0.47^{bc}(±0.11)	Z19	1.45^{b}(±0.20)	N7	0.35^{ab}(±0.02)
N10	9.2^{ab}(±0.60)	M74	7.7^{abc}(±0.33)	M29	16.9^{a}(±0.99)	N7	0.45^{bc}(±0.05)	N8	1.39^{b}(±0.10)	M125	0.35^{ab}(±0.05)
N7	9.0^{ab}(±0.62)	M32	7.3^{bcd}(±0.56)	M119	16.6^{a}(±2.05)	N8	0.40^{bc}(±0.04)	N7	1.31^{b}(±0.14)	M32	0.31^{ab}(±0.08)
M32	8.8^{ab}(±1.54)	M70	7.3^{bcd}(±0.38)	N8	16.4^{a}(±1.19)	N5	0.39^{bc}(±0.05)	M74	1.27^{b}(±0.31)	N8	0.30^{ab}(±0.03)
M74	8.3^{a}(±1.22)	Z5	6.7^{cd}(±0.35)	M82	16.4^{a}(±1.25)	M32	0.31^{bc}(±0.08)	M32	1.22^{b}(±0.20)	Z16	0.28^{ab}(±0.05)
M70	8.1^{a}(±1.16)	M19	6.3^{cd}(±0.26)	Z19	15.7^{a}(±1.52)	M74	0.29^{bc}(±0.11)	N10	1.19^{b}(±0.13)	M70	0.28^{ab}(±0.08)
Z5	7.8^{a}(±2.36)	N10	6.2^{cd}(±0.18)	N10	15.6^{a}(±0.96)	M70	0.27^{bc}(±0.04)	N5	0.96^{b}(±0.11)	M87	0.29^{ab}(±0.08)
M87	7.1^{a}(±1.46)	N5	6.1^{d}(±0.22)	M74	15.5^{a}(±1.54)	M87	0.21^{c}(±0.06)	M82	0.91^{b}(±0.18)	M82	0.23^{ab}(±0.04)
M119	6.9^{a}(±1.57)	N7	6.0^{d}(±0.19)	N5	15.2^{a}(±0.62)	M82	0.19^{c}(±0.04)	M119	0.82^{b}(±0.11)	M19	0.22^{ab}(±0.04)
N5	6.9^{a}(±0.78)	M119	5.9^{d}(±0.26)	M19	15.2^{a}(±0.81)	Z5	0.17^{c}(±0.09)	M19	0.75^{b}(±0.14)	M74	0.21^{ab}(±0.07)
M82	6.8^{a}(±0.97)	M82	5.8^{d}(±0.34)	M70	13.2^{a}(±0.78)	M19	0.17^{c}(±0.05)	Z5	0.70^{b}(±0.22)	Z5	0.21^{ab}(±0.06)
M19	6.5^{a}(±1.08)	N8	5.8^{d}(±0.19)	M32	13.0^{a}(±1.31)	M119	0.14^{c}(±0.06)	M87	0.69^{b}(±0.10)	M119	0.16^{b}(±0.06)

**/: RCD=root collar diameter, SDW=shoot dry weight, RDW=root dry weight*

[a]/: Means followed by the same letter(s) are not significantly different

(): Numbers in parentheses are standard errors